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- (54)Recombinant thermostable enzyme for converting maltose into trehalose...
- Disclosed are a recombinant thermostable enzyme, which converts maltose into trehalose and is stable up to a temperature of about 80°C even when incubated at pH 7.0 for 60 min, a preparation of the enzyme,
- a DNA encoding the enzyme, a recombinant DNA containing the DNA, a transformant, and an enzymatic conversion method of maltose by using the enzyme.

Description

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Background of th Inventi n

Field of the Invention

The present invention relates to a novel recombinant thermostable enzyme which converts maltose into trehalose.

Description of the Prior Art

Trehalose is a disaccharide which consists of 2 glucose molecules linked together with their reducing groups, and, naturally, it is present in bacteria, fungi, algae, insects, etc., in an extremely-small quantity. Having no reducing residue within the molecule, trehalose does not cause an unsatisfactory browning reaction even when heated in the presence of amino acids or the like, and because of this it can advantageously sweeten food products without fear of causing unsatisfactory coloration and deterioration. However, trehalose is far from being readily prepared in a desired amount by conventional methods, and, actually, it is not scarcely used for sweetening food products.

Conventional methods are roughly classified into 2 groups, i.e. the one using cells of microorganisms and the other employing a multi-enzymatic system wherein enzymes are allowed to act on saccharides. The former, as disclosed in Japanese Patent Laid-Open No.154,485/75, is a method which comprises allowing to grow microorganisms such as bacteria and yeasts in a nutrient culture medium, and collecting trehalose from the resultant culture. The latter, as disclosed in Japanese Patent Laid-Open No.216,695/83, is a method which comprises providing maltose as a substrate, allowing a multi-enzymatic system using maltose- and trehalose-phosphorylases to act on maltose, and isolating the formed trehalose from the reaction system. Although the former facilitates the growth of microorganisms without special difficulty, it has a drawback that the resultant culture only contains at most 15 w/w % trehalose, on a dry solid basis (d.s.b.). While the latter enables the separation of trehalose with a relative easiness, but it is theoretically difficult to increase the trehalose yield by allowing enzymes to act on substrates at a considerably-high concentration because the enzymatic reaction *per se* is an equilibrium reaction of 2 different types of enzymes and the equilibrium point constantly inclines to the side of forming glucose phosphate.

In view of the foregoing, the present inventors energetically screened enzymes which directly convert maltose into trehalose, and have found that microorganisms belonging to those of the genera *Pimelobacter* and *Pseudomonas*, as disclosed in Japanese Patent Application No.199,971/93, produce an absolutely novel enzyme which forms trehalose when acts on maltose. This means that trehalose can be prepared from maltose as a material which is readily available in quantity and at low cost, and the use of the enzyme would completely overcome all the aforesaid objects.

It was found that all the enzymes from these microorganisms have an optimum temperature of about 20-40°C which seems some how insufficient for trehalose production in their thermostability. It is recognized in this field that the saccharification of starch and amylaceous substances should be generally reacted at a temperature of over 55°C. If the saccharification reaction is effected at a temperature of 55°C or lower, bacterial contamination is enhanced to lower the pH of the reaction mixtures and to inactivate enzymes used, followed by remaining a relatively large amount of substrates intact. If the saccharification reaction is effected by using enzymes with poor thermostability, a great care should be taken for the pH changes, and, once a pH lowering occurs, alkalis should be quickly added to the reaction mixtures to increase the pH.

In view of the foregoing, the present inventors further studied on thermostable enzymes with such activity and have found that enzymes, produced from microorganisms of the genus *Thermus* such as a microorganism of the species *Thermus* aquaticus (ATCC 33923), effectively convert maltose into trehalose without being substantially inactivated even when reacted at a temperature of over 55°C. These enzymes, however, are not sufficient in enzyme producing activity, and this leads to a problem of that an industrial scale production of trehalose inevitably requires a considerably large scale cultivation of such microorganisms.

Recombinant DNA technology has made a remarkable progress in recent years. At present, even an enzyme, whose total amino acid sequence is not revealed, can be readily prepared in a desired amount, if a gene encoding the enzyme was once isolated and the base sequence was decoded, by preparing a recombinant DNA containing a DNA which encodes the enzyme, introducing the recombinant DNA into microorganisms or cells of plants or animals, and culturing the resultant transformants. Under these circumstances, urgently required are to find a gene encoding the above thermostable enzyme and to decode the base sequence.

Summary of the Invention

It is an object of the present invention to provide a recombinant thermostable enzyme which forms trehalose when acts on maltose.

It is a further object of the present invention to provide a DNA which encodes the recombinant enzyme.

It is yet another object of the present invention to provide a replicable recombinant DNA having the DNA.

It is a further object of the present invention to provide a transformant into which the recombinant DNA has been introduced.

It is a further object of the present invention to provide a process for preparing the recombinant enzyme by using the transformant.

It is a further object of the present invention to provide a method for converting maltose into trehalose by the recombinant enzyme.

The first object of the present invention is attained by a recombinant enzyme.

The second object of the present invention is attained by a DNA which encodes the recombinant enzyme.

The third object of the present invention is attained by a replicable recombinant DNA which contains the DNA and a self-replicable vector.

The fourth object of the present invention is attained by a transformant obtained by introducing the replicable recombinant DNA into an appropriate host.

The fifth object of the present invention is attained by culturing the transformant in a nutrient culture medium to form the recombinant enzyme, and collecting the formed recombinant enzyme from the resultant culture.

The sixth object of the present invention is attained by an enzymatic conversion method of maltose which contains a step of allowing the recombinant enzyme to act on maltose to form trehalose.

Brief Description of the Accompanying Drawings

FIG.1 shows the optimum temperature of an enzyme produced from Thermus aquaticus (ATCC 33923).

FIG.2 shows the optimum pH of an enzyme produced from Thermus aquaticus (ATCC 33923).

FIG.3 shows the thermal stability of an enzyme produced from Thermus aquaticus (ATCC 33923).

FIG.4 shows the pH stability of an enzyme produced from Thermus aquaticus (ATCC 33923).

FIG.5 shows the structure of the recombinant DNA pBTM22 according to the present invention.

FIG.6 shows the structure of the recombinant DNA pBTM23 according to the present invention.

Detailed Description of the Invention

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The recombinant enzyme according to the present invention acts on maltose to form trehalose without being substantially inactivated even when allowed to react at a temperature of over 55°C.

The DNA according to the present invention expresses the production of the present recombinant enzyme when introduced into an appropriate self-replicable vector to obtain a replicable recombinant DNA, then introduced into an appropriate host, which is inherently incapable of forming the recombinant enzyme but readily proliferative, to form a transformant

The recombinant DNA according to the present invention expresses the production of the recombinant enzyme by introducing it into an appropriate host, which is inherently incapable of forming the recombinant enzyme but readily proliferative, to form a transformant, and culturing the transformant in a nutrient culture medium.

The transformant forms a desired amount of the recombinant enzyme when cultured according to the present invention.

The enzymatic conversion method according to the present invention converts maltose into a saccharide composition comprising trehalose, glucose and/or maltooligosaccharides.

The present invention was made based on the finding of an absolutely novel thermostable enzyme which converts maltose into trehalose. Such an enzyme can be obtained from cultures of *Thermus aquaticus* (ATCC 33923), and the present inventors isolated the enzyme by using a variety of methods comprising column chromatography as a main technique, and studied on the properties and features, revealing that the reality is a polypeptide having the following physicochemical properties:

(1) Action

Forming trehalose when acts on maltose, and vice versa;

(2) Molecular weight (MW)

About 100,000-110,000 daltons when assayed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE);

(3) Isoelectric point (pl)

About 3.8-4.8 when assayed on isoelectrophoresis;

(4) Optimum temperature

About 65°C when incubated at pH 7.0 for 60 min;

(5) Optimum pH

About 6.0-6.7 when incubated at 60°C for 60 min;

(6) Thermal stability

Stable up to a temperature of about 80°C even when incubated at pH 7.0 for 60 min; and

(7) pH Stability

Stable up to a pH of 5.5-9.5 even when incubated at 60°C for 60 min.

Experiments for revealing the physicochemical properties of a thermostable enzyme produced from *Thermus aquaticus* (ATCC 33923) are as follows:

Experiment 1

Purification of enzyme

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Experiment 1-1

Production of enzyme

In 500-ml Erlenmeyer flasks were placed 100 ml aliquots of a liquid culture medium (pH 7.5) containing 0.5 w/v % polypeptone, 0.1 w/v % yeast extract, 0.07 w/v % sodium nitrate, 0.01 w/v % disodium hydrogen phosphate, 0.02 w/v % magnesium sulfate heptahydrate, 0.01 w/v % calcium chloride, and water, and the flasks were autoclaved at 120°C for 20 min to effect sterilization. After cooling the flasks a seed culture of *Thermus aquaticus* (ATCC 33923) was inoculated into each flask, followed by the incubation at 60°C for 24 hours under a rotary-shaking condition of 200 rpm to obtain a seed culture. Twenty L aliquots of a fresh preparation of the same liquid culture medium were put in 30-L jar fermenters, sterilized and cooled to 60°C, followed by inoculating one v/v % of the seed culture into each fermenter, and incubating the resultant at a pH of 6.0-8.0 and 60°C for about 20 hours under aeration-agitation conditions.

Thereafter, the enzymatic activity of the resultant culture was assayed to reveal that it contained about 0.35 units/ml of the enzyme. A portion of the culture was centrifuged, and the supernatant was assayed to reveal that it contained about 0.02 units/ml of the enzyme. While the separated cells were suspended in 50 mM phosphate buffer (pH 7.0) to give the total volume equal to the original volume of the portion, followed by assaying the suspension to reveal that it contained about 0.33 units/ml of the enzyme.

Throughout the specification the enzyme activity is expressed by the value measured on the following assay: Place one ml of 10 mM phosphate buffer (pH 7.0) containing 20 w/v % maltose in a test tube, add one ml of an appropriately diluted enzyme solution to the tube, and incubate the solution in the tube at 60°C for 60 min to effect an enzymatic reaction, followed by a further incubation at 100°C for 10 min to suspend the enzymatic reaction. Thereafter, a portion of the reaction mixture was diluted by 11 times with 50 mM phosphate buffer (pH 7.5), and 0.4 ml of which was placed in a test tube, admixed with 0.1 ml solution containing one unit/ml trehalase, followed by incubating the resultant mixture at 45°C for 120 min and quantifying the glucose content on the glucose oxidase method. As a control, a system using a trehalase solution and an enzyme solution which has been inactivated by heating at 100°C for 10 min is provided and treated similarly as above. The content of the formed trehalose is estimable based on the content of glucose quantified in the above. One unit of the enzyme activity is defined as the amount which forms one µmol trehalose per min under the above conditions.

45 Experiment 1-2

Purification of enzyme

The culture obtained in Experiment 1-1 was centrifuged to separate cells, and about 0.28 kg of the wet cells thus obtained was suspended in 10 mM phosphate buffer (pH 7.0), disrupted in usual manner, and centrifuged to obtain an about 1.8 L of a crude enzyme solution. The solution was admixed with ammonium sulfate to give a saturation of 70 w/v %, salted out by standing at 4°C overnight, and centrifuged to obtain a supernatant. The supernatant was mixed with 10 mM phosphate buffer (pH 7.0), and the mixture solution was dialyzed against a fresh preparation of the same buffer for 24 hours.

The dialyzed inner solution was centrifuged to obtain a supernatant (1,560 ml) which was then applied to a column packed with 530 ml of "DEAE-TOYOPEARL® 650", an ion exchanger commercialized by Tosoh Corporation, Tokyo, Japan, which had been previously equilibrated with 10 mM phosphate buffer (pH 7.0), followed by feeding to the column a linear gradient buffer of sodium chloride ranging from 0 M to 0.4 M in 10 mM phosphate buffer (pH 7.0). From the

eluate, fractions with the objective enzyme activity were collected, pooled, dialyzed against 10 mM phosphate buffer (pH 7.0) containing one M ammonium sulfate for 10 hours, and centrifuged to obtain a supermatant. The supernatant was applied to a column packed with 380 ml of "BUTYL-TOYOPEARL® 650", a gel for hydrophobic chromatography commercialized by Tosoh Corporation, Tokyo, Japan, which had been previously equilibrated with 10 mM phosphate buffer (pH 7.0) containing one M ammonium sulfate, followed by feeding to the column a linear gradient buffer of ammonium sulfate ranging from 1 M to 0 M in 10 mM phosphate buffer (pH 7.0).

Fractions, eluted at 0.2 M ammonium sulfate, with the objective enzyme activity were collected, pooled and dialyzed against 10 mM phosphate buffer (pH 7.0) containing 0.2 M sodium chloride for 16 hours. The dialyzed solution was centrifuged to remove insoluble substances, fed to a column packed with 380 ml of "TOYOPEARL® HW-55S", a gel for gel filtration chromatography commercialized by Tosoh, Corporation, Tokyo, Japan, which had been previously equilibrated with 10 mM phosphate buffer (pH 7.0) containing 0.2 M sodium chloride, followed by feeding to the column with 10 mM phosphate buffer (pH 7.0) containing one M sodium chloride. Fractions with the enzyme activity were collected from the eluate, fed to a column packed with "MONO Q HR5/5" which had been equilibrated with 10 mM phosphate buffer (pH 7.0). The column was fed with a linear gradient buffer of sodium chloride ranging from 0.1 M to 0.35 M in 10 mM phosphate buffer (pH 7.0), followed by collecting fractions with the enzyme activity. The purified enzyme thus obtained had a specific activity of about 135 units/mg protein in a yield of about 330 units per L of the culture.

The purified enzyme was electrophoresed in a 7.5 w/v % polyacrylamide gel to give a single protein band with the enzyme activity, and this meant that it had a considerably-high purity.

Experiment 2

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Physicochemical property of enzyme

Experiment 2-1

<u>Action</u>

To an aqueous solution containing 5 w/w % maltose or trehalose as a substrate was added 2 units/g substrate of the purified enzyme obtained in Experiment 1-2, and the mixture was incubated at 60°C and pH 7.0 for 24 hours. In order to analyze the saccharide composition of the reaction mixture, it was dried *in vacuo*, dissolved in pyridine, and trimethylsilylated in usual manner, and the resultant was subjected to gas chromatography. The equipments and conditions used in this analysis were as follows: "GC-16A" commercialized by Shimadzu Seisakusho, Ltd., Tokyo, Japan, as a gas chromatograph; a stainless steel column, having an inner diameter of 3 mm and a length of 2 m, packed with 2% "SILICONE OV-17/CHROMOSOLB W" commercialized by GL Sciences Inc., Tokyo, Japan, as a column; a hydrogen flame type of ionization as a detector; nitrogen gas as a carrier gas (flow rate of 40 ml/min); and a column oven temperature of 160-320°C at a programmed increasing temperature rate of 7.5°C/min. The saccharide compositions of the reaction mixtures were tabulated in Table 1:

Table 1

Substrate	Saccharide com	position of reacti	on mixture (%)
	Trehalose	Glucose	Maltose
Maltose	70.0	4.4	25.6
Trehalose	76.2	3.1	20.7

As is shown in Table 1, the purified enzyme formed about 70 w/w % trehalose and about 4 w/w % glucose when acted on maltose as a substrate, while it formed about 21 w/w % maltose and about 3 w/w % glucose when acted on trehalose as a substrate. These facts indicate that the purified enzyme has activities of converting maltose into trehalose and of converting trehalose into maltose, as well as of hydrolyzing α l,4 linkage in maltose molecule and α , α -1,1 linkage in trehalose molecule. There has been no report of such an enzyme, and this leads to an estimation of having a novel enzymatic pathway.

Experiment 2-2

Molecular weight

In accordance with the method as reported by U. K. Laemmli in Nature, Vol.227, pp.680-685 (1970), the purified

enzyme was electrophoresed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to give a single protein band at a position corresponding to about 100,000110,000 daltons. The marker proteins used in this experiment were myosin (MW=200,000 daltons), β-galactosidase (MW=116,250 daltons), phosphorylase B (MW=97,400 daltons), serum albumin (MW=66,200 daltons) and ovalbumin (MW=45,000 daltons).

Experiment 2-3

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Isoelectric point

The purified enzyme gave an isoelectric point of about 3.8-4.8 when isoelectrophoresed in 2 w/v % "AMPHOLINE®", a polyacrylamide gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden.

Experiment 2-4

15 Optimum temperature

The optimum temperature of the purified enzyme was about 65°C as shown in FIG.1 when incubated in usual manner in 10 mM phosphate buffer (pH 7.0) for 60 min.

20 Experiment 2-5

Optimum pH

The optimum pH of the purified enzyme was about 6.0-6.7 as shown in FIG.2 when tested in usual manner by incubating it at 60°C for 60 min in 10 mM acetate buffer, phosphate buffer or sodium carbonate/sodium hydrogen carbonate buffer with different pHs.

Experiment 2-6

30 Thermal stability

The purified enzyme was stable up to a temperature of about 80°C as shown in FIG.3 when tested in usual manner by incubating it in 50 mM phosphate buffer (pH 7.0) for 60 min.

35 Experiment 2-7

pH Stability

The purified enzyme was stable up to a pH of about 5.5-9.5 as shown in FIG.4 when experimented in usual manner by incubating it at 60°C for 60 min in 50 mM acetate buffer, phosphate buffer or sodium carbonate/sodium hydrogen carbonate buffer with different pHs.

Experiment 2-8

45 Amino acid sequence containing the N-terminus

The amino acid sequence containing the N-terminus of the purified enzyme was analyzed on "MODEL 470A", a gas-phase protein sequencer commercialized by Perkin-Elmer Corp., Instrument Div., Norwalk, USA, and revealed to have the amino acid sequence containing the N-terminus in SEQ ID NO:1.

SEQ ID NO:1:

Met Asp Pro Leu Trp Tyr Lys Asp Ala Val Ile Tyr Gln Leu His Val 1 5 10 15 Arg Ser Phe Phe

Experiment 2-9

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Partial amino acid sequence

An adequate amount of the purified enzyme prepared in Experiment 1-2 was weighed, dialyzed against 10 mM Tris-HCl buffer (pH 9.0) at 4°C for 18 hours, and admixed with 10 mM Tris-HCl buffer (pH 9.0) to obtain a solution containing about one mg/ml of the enzyme. The solution was incubated at 100°C for 5 min to denature the enzyme, and about one ml of which was placed in a test tube, admixed with 40 µg lysyl endopeptidase, and incubated at 30°C for 44 hours to partially hydrolyze the enzyme. The resultant hydrolysate was applied to "µBONDASPERE C18", a column for reverse-phase high-performance liquid chromatography commercialized by Japan Millipore Ltd., Tokyo, Japan, which had been equilibrated with 0.1 v/v % trifluoroacetate, followed by feeding to the column 0.1 v/v % trifluoroacetate containing acetonitrile at a flow rate of 1.0 ml/min while increasing the concentration of acetonitrile from 0 v/v % to 70 v/v %.

Fractions containing a peptide fragment eluted about 58 min to 60 min after the initiation of the feeding were collected, pooled, dried *in vacuo*, and dissolved in 0.5 ml of 10 mM Tris-HCl buffer (pH 8.0), admixed with 5 µg TPCK treated trypsin, and incubated at 37° C for 16 hours to effect hydrolysis. The enzymatic reaction was suspended by freezing, and the resultant hydrolyzate was fed to a column packed with "µBONDASPERE C18", followed by feeding to the column 0.1 v/v % trifluoroacetate containing aqueous acetonitrile at a flow rate of 1.0 ml/min while increasing the concentration of aqueous acetonitrile from 15 v/v % to 55 v/v %. Fractions, containing a peptide fragment eluted about 42 min after the initiation of the feeding, were collected, pooled, dried *in vacuo*, and dissolved in 0.1 v/v trifluoroacetate containing 50 v/v % aqueous acetonitrile. Similarly as in Experiment 2-8, it was revealed that the peptide fragment contained the amino acid sequence in SEQ ID NO:2.

SEQ ID NO:2:

Since no enzyme with these physicochemical properties has been known, it can be estimated to be a novel substance

The present inventors energetically screened the chromosomal DNA of *Thermus aquaticus* (ATCC 33923) by using an oligonucleotide as a probe which had been chemically synthesized based on the amino acid sequences as revealed in Experiments 2-8 and 2-9, and have obtained a DNA fragment which consisted of about 3,600 base pairs having the base sequence in SEQ ID NO.4. The decoding of the base sequence revealed that a thermostable enzyme from the microorganism consists of 963 amino acids and has the amino acid sequence in SEQ ID NO.3.

SEQ ID NO:3:

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15	Phe				25					90					Ile 95	
				100					100					110	Glu	
			115					-120					123		Asp	
20		720					1.33					7-20			Glu	
	4 4 5					150					133				His Val	100
25					165					1/0					Val 175 Val	
	_			1 20					TRD					730	Val Gly	
			105					-200					203		Leu	
30		0 . 0					215					220			Ala	
	225					7311					200				Gly 255	
35					245					250					255 Phe	
				260					200					4,70	Lys	
			つつに					- 280					200		Arg	
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45					375				Leu	Gly				Arg	Arg	
				340				Leu	Thr	;			Thr	Pro	Ile	
	Tyr	Tyr	355 Gly	Asp	Glu	Ile	Gly	360 Met	Gly	Asp	Asn	Pro	365 Phe	Leu	Gly	Asp
50			Gly	Val	Arg	Thr	375 Pro	Met	Gln	Trp	Ser 395	380 Gln		Arg	Ile	Val 400
	385 Ala	Phe	Ser	Arg	Ala	390 Pro	Tyr	His	Ala	Leu	Phe	Leu	Pro	Pro	Val 415	Ser
55	Glu	Gly	Pro	Tyr 420		Tyr	His	: Phe	val 425	410 Asn	val	Glu	Ala	430	Arg	

			435					440					445	Ala		
		450					455					460		Leu		
5	465	Glu		_		470					475			Glu		480
•	Arg	Val	Leu	Val	Val 485	Ala	Asn	Leu	Ser	Arg 490	Tyr	Thr	Gln	Ala	Phe 495	Asp
10				500					505					Leu 510		
	•		515					520					525	Thr		
		530	_				535					540		Ala		
15	545					550					555			Glu		560
	Asp				565					570				Leu	575	
20				580					585					Asn 590		
			595					600					605	Pro		
		610					615					620		Pro		
25	625					630					635			Val		640
					645					650				Leu	655	
30	_			660					665					Leu 670		
			675					680					685	Phe		
,	_	690					695					700			0	Pro ·
35	705					710					715	•		Glu	-	720
	_				725					730				Arg	735	
40				740					745					Pro 750		
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4 5		770					775					780		Ala		
	785					790					795			Ala		800
					805					810				Ala	815	•
50	_			820					825					Leu 830		
			835					840					845	Gly		
55		850					855					860		Leu		
	Glu	Lys	Arg	Gly	Thr	Val	Glu	Glu	Asp	Leu	Ala	Arg	Leu	Ala	TYT	ASP

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870
               865
               Val Glu Arg Ala Val His Leu Ala Leu Glu Ala Leu Glu Ala Glu Leu
               885 890 895
Trp Ala Phe Ala Glu Glu Val Ala Asp His Leu His Ala Ala Phe Leu
                                                                                                                                                                                   910
                                                                                                                   905
                                                      900
                Gln Ala Tyr Arg Ser Ala Leu Pro Glu Glu Ala Leu Glu Glu Ala Gly
                                                                                                                                                                    925
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                Trp Thr Arg His Met Ala Glu Val Ala Ala Glu His Leu His Arg Glu
                930 935 940
Glu Arg Pro Ala Arg Lys Arg Ile His Glu Arg Trp Gln Ala Lys Ala
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                                                                                                                                             955
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                Gly Lys Ala
            SEQ ID NO:4:
15
            GTGGACCCCC TCTGGTACAA GGACGCGGTG ATCTACCAGC TCCACGTCCG CTCCTTCTTT
            GACGCCAACA ACGACGGCTA CGGGGACTTT GAGGGCCTGA GGCGGAAGCT TCCCTACCTG
GAGGAGCTCG GGGTCAACAC CCTCTGGCTC ATGCCCTTCT TCCAGTCCCC CTTGAGGGAC
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            GACGGGTACG ATATCTCCGA CTACTACCAG ATCCTCCCCG TCCACGGGAC CCTGGAGGAC
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            TTCACCGTGG ACGAGGCCA CGGCCGGGGG ATGAAGGTGA TCATTGAGCT CGTCCTGAAC CACACCTCCA TTGACCACC TTGGTTCCAG GAGGCGAGGA AGCCGAATAG CCCCATGCGG GACTGGTACG TGTGGAGCGA CACCCCGGAG AAGTACAAGG GGGTCCGGGT CATCTTCAAG
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             GACTTTGAAA CCTCCAACTG GACCTTTGAC CCCGTGGCCA AGGCCTACTA CTGGCACCGC
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             TTCTACTGGC ACCAGCCCGA CCTCAACTGG GACAGCCCCG AGGTGGAGAA GGCCATCCAC
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             TTCCTCCGCA ACCACGACGA GCTCACCCTG GAGAAGGTCA CGGAGGAGGA GCGGGAGTTC
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             ACCUCTAAAGG GCACGCCCAT CGTCACTAC GGGGACGAGA TCGGCATGGC CCGCATCGCC 1200
TTCCTCGGGG ACCGGAACGG TGTCAGGACC CCCATGCAGA CCGCATCGTC 1200
GCCTTCTCCC GCGCCCCTA CCACGCCCCT TTCCTTCCCC CCGTGAGCGA GGGCCCTAC 1260
ACCGCCGCT TCGTCAACGT GGAGGACCAG CGCGCAAAACC CCCACTCCCT CCTGAGCTTC 1320
AACCGCCGCT TCCTCGCCCT GAGGAACCAG CACGCCCAAGA TCTTCGGCCG GGGGAGCCTC 1380
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CACGAAAGGG GGCGGAGGA GCCCCTCCACCT AGACCCTGAA GGAAAGAGAC 1800
TGGCTCGCCC TCAAGCCGCA GAAGGTGGCC CTCCTGGACG CCCTCCGCTT CCAGAAGGAC 1860
              CCGCCCTTT ACCTCACCT GCTCCAGCTG GAGAACCACA GGACCCTCCA GGTCTCCTC 1920
CCCCTCCTCT GGTCCCCCA GAGGCGGGAA GGCCCCGGCC TCTTCGCCCG CACCCACGGC 1980
              CAGCCCGGCT ACTTCTACGA GCTCTCCTTG GACCCAGGCT TCTACCGCCT CCTCCTCGCC 2040
              CGCCTTAAGG AGGGGTTTGA GGGGCGGAGC CTCCGGGCCT ACTACCGCGG CCGCCACCCG 2100
              GGTCCCGTGC CCGAGGCCGT GGACCTCCTC CGGCCGGGAC TCGCGGCGGG GGAGGGGGTC 2160
TGGGTCCAGC TCGCCCTCGT CCAAGACGGG GGCCTGGACC GCACGGAGCG GGTCCTCCCC 2220
CGCCTGGACC TCCCCTGGGT TCTCCGGCCC GAAGGGGGCC TCTTCTGGGA GCGGGGCGC 2280
TCCAGAAAGGG TCCTCGCCCT CACGGGAAGC CTCCCCCCGG GCCGCCCCCA GGACCTCTTC 2340
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              GCCGCCCTGG AGGTCCGGCT CCTGGAAAGC CTTCCCCGCC TCCGGGGGCA CGCCCCGGG 2400
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GTGCGCCTCG CCCCTCCTAG GCCCTGGGCG GTGGAGCGGG GAGGAGGTGG GAGGAGGCCC CTCCACCGGG	TCCTTCCGG CCCTCCTCA GCCGCGGCCT CGGAAAAGCG CCGTGCACCT CCGACCACCT TGGAGGAGGC AGGAAAGGCC	CCGGGCCCTT CGGGGCCTTC GGGCACGGTG CGCCTCGAG CCACGCCGCC	GGGGAGGTGG CTGGAGCTGG GAGGAGGACC GCCCTGGAGG TTCCTCCAAG	AGGGGGTGGT AGGGGGGAGCTTTG CCTACCGCTC CCGAGGTGGC	GTACCTCGTG GGCCTACGAC GGCCTTTGCC CGCCCTCCCC GGCGGAGCAC	2580 2640 2700 2760 2820
GGAAAAGCC						

The sequential experimental steps used to reveal the amino acid sequence and the base sequence in SEQ ID NOs:3 and 4 are summarized in the below:

- (1) A thermostable enzyme was isolated from a culture of a donor microorganism, highly purified, and determined for its amino acid sequence containing the N-terminus. The purified enzyme was partially hydrolyzed with protease, and from which a peptide fragment was isolated and determined for its amino acid sequence;
- (2) Separately, a chromosomal DNA was isolated from a donor microorganism's cell, purified and partially digested with a restriction enzyme to obtain a DNA fragment consisting of about 4,000-8,000 base pairs. The DNA fragment was ligated with a DNA ligase to a plasmid vector, which had been previously cut with a restriction enzyme, to obtain a recombinant DNA;
- (3) The recombinant DNA was introduced into a microorganism of the species *Escherichia coli* to obtain transformants, and from which an objective transformant containing a DNA encoding the thermostable enzyme was selected by the colony hybridization method using an oligonucleotide, as a probe, which had been chemically synthesized based on the aforesaid partial amino acid sequence; and
- (4) The recombinant DNA was obtained from the selected transformant and annealed with a primer, followed by allowing a DNA polymerase to act on the resultant to extend the primer, and determining the base sequence of the resultant complementary chain DNA by the dideoxy chain termination method. The comparison of an amino acid sequence, which could be estimated based on the determined base sequence, with the aforesaid amino acid sequence concluded that it encodes the thermostable enzyme.

The following Experiments 3 and 4 concretely illustrate the above items (2) to (4), and the techniques used therein were conventional ones commonly used in this field, for example, those described by J. Sumbruck et al. in "Molecular Cloning A Laboratory Manual", 2nd edition, published by Cold Spring Harbor Laboratory Press (1989).

35 Experiment 3

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Preparation of recombinant DNA containing DNA encoding thermostable enzyme, and transformant

Experiment 3-1

Preparation of chromosomal DNA

A seed culture of *Thermus aquaticus* (ATCC 33923) was inoculated into nutrient broth medium (pH 7.0), and cultured at 60°C for 24 hours with a rotary shaker. The cells were separated from the resultant culture by centrifugation, suspended in TES buffer (pH 8.0), admixed with 0.05 w/v % lysozyme, and incubated at 37°C for 30 min. The resultant was freezed at -80°C for one hour, admixed with TSS buffer (pH 9.0), heated to 60°C, and further admixed with a mixture solution of TES buffer and phenol, and the resultant solution was chilled with ice, followed by centrifugation to obtain a supernatant. To the supernatant was added 2-fold volumes of cold ethanol, and the precipitated crude chromosomal DNA was collected, suspended in SSC buffer (pH 7.1), admixed with 7.5 µg ribonuclease and 125 µg protease, and incubated at 37°C for one hour. Thereafter, a mixture solution of chloroform and isoamyl alcohol was added to the reaction mixture to extract the objective chromosomal DNA, and the extract was admixed with cold ethanol, followed by collecting the formed sediment containing the chromosomal DNA. The resultant purified chromosomal DNA was dissolved in SSC buffer (pH 7.1) to give a concentration of about one mg/ml, and the resultant solution was freezed at -80°C.

Experiment 3-2

Preparation of recombinant DNA pBTM22 and transformant BTM22

About one ml of the purified chromosomal DNA obtained in Example 3-1 was placed in a test tube, admixed with about 10 units of *Sau* 3Al, a restriction enzyme, and enzymatically reacted at 37°C for about 20 min to partially cleave the chromosomal DNA, followed by recovering a DNA fragment consisting of about 4,000-8,000 base pairs by means of sucrose density-gradient ultracentrifugation. One µg of Bluescript II SK(+), a plasmid vector commercialized by Stratagene Cloning Systems, California, USA, was placed in a test tube, subjected to the action of *Bam* HI, a restriction enzyme, to completely digest the plasmid vector, admixed with 10 µg of the DNA fragment and 2 units of T4 DNA ligase, and allowed to stand at 4°C overnight to ligate the DNA fragment to the plasmid vector fragment. To the resultant recombinant DNA was added 30 µl of "Epicurian Coli® XLI-Blue", a competent cell commercialized by Stratagene Cloning Systems, California, USA, Japan, allowed to stand under ice-chilling conditions for 30 min, heated to 42°C, admixed with SOC broth, and incubated at 37°C for one hour to introduce the recombinant DNA into *Escherichia coli*.

The resultant transformant was inoculated into agar plate (pH 7.0) containing 50 μg/ml of 5-bromo-4-chloro-3-in-dolyl-β-galactoside, and cultured at 37°C for 18 hours, followed by placing a nylon film on the agar plate to fix thereon about 6,000 colonies formed on the agar plate. Based on the amino acid sequence of Trp-Tyr-Lys-Asp-Ala-Val as shown in SEQ ID NO:1, the base sequence of probe 1 represented by the base sequence of 5'-TGGTAYAARGAYGCNGT-3' was chemically synthesized, labelled with ³²P, and hybridized with the colonies of transformants fixed on the nylon film, followed by selecting 5 transformants which had strongly hybridized with the probe 1.

The objective recombinant DNA was selected in usual manner from the 5 transformants, and, in accordance with the method described by E. M. Southern in *Journal of Molecular Biology*, Vol.98, pp.503-517 (1975), the-recombinant DNA was hybridized with probe 2 represented by the base sequence of 5'-AAYATGTGGCCNGARGA-3', which had been chemically synthesized based on the amino acid sequence in SEQ ID NO:2, i.e. Asn-Met-Trp-Pro-Glu-Glu, and labelled with ³²P, followed by selecting a recombinant DNA which had strongly hybridized with the probe 2. The recombinant DNA and the transformant thus selected were respectively named "pBTM22" and "BTM22".

The transformant BTM22 was inoculated into L-broth (pH 7.0) containing 100 µg/ml ampicillin, and cultured at 37°C for 24 hours by a rotary shaker. After completion of the culture, the resultant cells were centrifugally collected from the culture, and treated with conventional alkaline method to extract a recombinant DNA from the cells. The extract was in usual manner purified and analyzed and revealing that the recombinant DNA pBTM22 consists of about 10,300 base pairs. As is shown in FIG.5, a fragment containing a DNA, which consists of about 2,900 base pairs and encodes the thermostable enzyme, is located in the downstream near the digested site of *Hind* III, a restriction enzyme.

Experiment 3-3

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Production of recombinant enzyme by transformant BTM22

In 500-ml flasks were placed 100 ml aliquots of a liquid nutrient culture medium (pH 7.0) consisting of 2.0 w/v % glucose, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % dipotassium hydrogen phosphate, 0.06 w/v % sodium dihydrogen phosphate, 0.05 w/v % magnesium sulfate heptahydrate, 0.5 w/v % calcium carbonate and water, and each flasks was sterilized by heating at 115°C for 30 min, cooled, admixed with 50 µg/ml ampicillin, and inoculated with the transformant BTM22 obtained in Experiment 3-2, followed by culturing the transformant at 37°C for 24 hours by a rotary shaker. The resultant culture was treated with an ultrasonic disintegrator to disrupt cells, and the resultant suspension was centrifuged to remove insoluble substances. The supermatant thus obtained was assayed for the enzyme activity and revealing that one L of the culture contained about 800 units of a recombinant enzyme.

As a control, a seed culture of *Escherichia coli* XLI-Blue or *Thermus aquaticus* (ATCC 33923) was inoculated in a fresh preparation of the same liquid nutrient culture medium but free of ampicillin, and, in the case of culturing *Thermus aquaticus* (ATCC 33923), it was cultured and treated similarly as above except that the cultivation temperature was set to 65°C. Assaying the activity of the resultant, one L culture of *Thermus aquaticus* contained about 350 units of the enzyme, and the yield was significantly lower than that of transformant BTM22. *Escherichia coli* XLI-Blue used as a host did not form the thermostable enzyme.

Thereafter, the enzyme produced by the transformant BTM22 was purified similarly as in Experiments 1 and 2, and examined for its physicochemical properties and features. As a result, it was revealed that it has substantially the same physicochemical properties as the thermostable enzyme of *Thermus aquaticus* (ATCC 33923), i.e. it has a molecular weight of about 100,000-110,000 daltons on SDS-PAGE and an isoelectric point of about 3.8-4.8 on isoelectrophoresis, and is not substantially inactivated even when incubated at 80°C for 60 min in water (pH 7.0). The results indicate that the present thermostable enzyme can be prepared by recombinant DNA technology, and the yield can be significantly increased thereby.

Experiment 4

Preparation of complementary chain DNA and determination for its base sequence and amino acid sequence

Two μg of the recombinant DNA pBTM22 in Experiment 3-2 was placed in a test tube, admixed with 2 M aqueous sodium hydroxide solution to effect degeneration, and admixed with an adequate amount of cold ethanol, followed by collecting the formed sediment containing a template DNA and drying the sediment *in vacuo*. To the template DNA were added 50 pmole/ml of a chemically synthesized primer represented by the base sequence of 5'-GTAAAACGACG-GCCAGT-3', 10 μ l of 40 mM Tris-HCl buffer (pH 7.5) containing 20 mM magnesium chloride and 20 mM sodium chloride, and the mixture was incubated at 65°C for 2 min to effect annealing and admixed with 2 μ l of an aqueous solution containing dATP, dGTP and dTTP in respective amounts of 7.5 μ M, 0.5 μ l of [α -32P]dCTP (2 mCi/ml), one μ l of 0.1 M dithiothreitol, and 2 μ l of 1.5 units/ml T7 DNA polymerase, followed by incubating the resultant mixture at 25°C for 5 min to extend the primer from the 5'-terminus to the 3'-terminus. Thus, a complementary chain DNA was formed.

The reaction product containing the complementary chain DNA was divided into four equal parts, to each of which 2.5 µl of 50 mM aqueous sodium chloride solution containing 80 µM dNTP and 8 µM ddATP, ddCTP, ddGTP or ddTTP was added, and the resultant mixture was incubated at 37°C for 5 min, followed by suspending the reaction by the addition of 4 µl of 98 v/v % aqueous formamide solution containing 20 mM EDTA, 0.05 w/v % bromophenol blue, and 0.05 w/v % xylene cyanol. The reaction mixture was heated with a boiling-water bath for 3 min, and a small portion of which was placed on a 6 w/v % polyacrylamide gel, and electrophoresed by energizing it with a constant voltage of about 2,000 volts to separate DNA fragments, followed by fixing the gel in usual manner, drying it and subjecting the resultant to autoradiography.

Analyses of the DNA fragments separated on the radiogram revealed that the complementary chain DNA contains the base sequence consisting of about 3,600 base pairs in SEQ ID NO:5. An amino acid sequence estimable from the base sequence was as shown in parallel in SEQ ID NO:5, and it was compared with the amino acid sequence containing the N-terminus or the partial amino acid sequences in SEQ ID NOs:1 and 2 and revealing that the amino acid sequence in SEQ ID NO:1 corresponded to that positioning from 1 to 20 in SEQ ID NO:5, and the amino acid sequence in SEQ ID NO:2 corresponded to that positioning from 236 to 250 in SEQ ID NO:5. These results indicate that the present recombinant enzyme has the amino acid sequence in SEQ ID NO:3, and the amino acid sequence of the DNA derived from *Thermus aquaticus* (ATCC 33923) is encoded by the base sequence in SEQ ID NO:4.

SEQ ID NO:5:

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GCCCTCCT CCCCAACCG TGCTCGACGG GGAGGTGCGG CTTCCCTCT ACCCCGGGT CGGGGGCGG AGGGCAGGA GAGGGGCTCC TGGGGGCCTT AAGCCCAAGG GGGTGGACAA	CCCCTCTTGC GCGGGTGGAG GAAGGCCCGG GGGCCTCGAG	GCCGTGGGCC GACAAGGGCT GCCTGCCTCG	TCGCCCTGGC AGGCCTGGCT GCAAGAGGGT	CCTGCACTAC TAAGGCGGTG CCTGGAGCTC	60 120 180 240 300 360
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5	GTG	GAC	CCC	CTC	TGG	TAC Tyr	AAG	GAC	GCG	GTG	ATC	TAC	CAG	CTC	CAC	GTC	588
	CGC	TCC Ser	TTC Phe	TTT Phe 20	GAC Asp	GCC Ala	AAC Asn	AAC Asn	GAC Asp 25	GGC Gly	TAC Tyr	GGG Gly	GAC Asp	TTT Phe 30	GAG Glu	Gly	636
10	CTG Leu	AGG Arg	CGG Arg 35	AAG	CTT Leu	CCC Pro	TAC Tyr	CTG Leu 40	GAG	GAG Glu	CTC Leu	GGG Gly	GTC Val 45	AAC Asn	ACC Thr	CTC Leu	684
46	TGG Trp	CTC Leu	ATG	CCC Pro	TTC Phe	TTC Phe	Gln	TCC Ser	CCC Pro	TTG Leu	AGG Arg	Asp	GAC Asp	GGG Gly	TAC Tyr	GAT Asp	732
15	ATC	50 TCC	GAC	TAC	TAC	CAG Gln	55 ATC	CTC	CCC	GTC	CAC	GGG	ACC	CTG	GAG	GAC	780
	65 TTC	ACC	GTG	GAC	GAG	70 GCC	CAC	GGC	CGG	GGG	75 ATG	AAG	GTG	ATC	ATT	80 GAG	828
20	Phe	Thr	Val	Asp	Glu 85	Ala	His	Gly	Arg	Gly 90	Met	Lys	Val	Ile	Ile 95	Glu	0.00
	CTC Leu	GTC Val	CTG Leu	AAC Asn 100	His	ACC Thr	Ser	Ile	Asp 105	His	Pro	Trp	Phe	Gln 110	Glu	Ala	876
25	AGG Arg	AAG Lys	Pro	AAT	AGC Ser	CCC Pro	ATG Met	Arg	GAC	TGG Trp	TAC Tyr	GTG Val	Trp	AGC Ser	GAC Asp	ACC Thr	924
	CCG	GAG	AAG	TAC	AAG	GGG Gly	GTC	CGG Arg	GTC Val	ATC	TTC	AAG Lvs	GAC Asp	TTT Phe	GAA Glu	ACC Thr	972
30	TCC	130 AAC	TGG	ACC	TTT	GAC	135 CCC	GTG	GCC	AAG	GCC	140 TAC	TAC	TGG	CAC	CGC	1020
	Ser 145	Asn	Trp	Thr	Phe	Asp 150	Pro	Val	Ala	Lys	Ala 155	Tyr	Tyr	Trp	His	Arg 160	1069
	TTC Phe	TAC	TGG	His	Gln 165	CCC Pro	Asp	Leu	Asn	Trp 170	Asp	Ser	Pro	Glu	Val 175	Glu	1068
35	AAG Lys	GCC Ala	ATC Ile	CAC His 180	CAG	GTC Val	ATG Met	TTC Phe	TTC Phe 185	TGG	GCC Ala	GAC Asp	CTG Leu	GGG Gly 190	GTG Val	GAC Asp	1116
40	GGC Gly	TTC Phe	Arg	CTG	GAC Asp	GCC Ala	ATC Ile	Pro	TAC	CTC Leu	TAC Tyr	GAG Glu	CGG Arg 205	GAG Glu	Gly GGG	ACC Thr	1164
**	TCC Ser	TGC Cys	195 GAG Glu	AAC Asn	CTC Leu	CCC	GAG Glu	ACC Thr	ATT Ile	GAG Glu	GCG Ala	GTG Val	AAG	CGC Arg	CTG Leu	AGG Arg	1212
	AAG	210 GCC	CTG	GAG	GAG	CGC	215 TAC	GGC	CCC	GGG	AAG	ATC	CTC	CTC	GCC	GAG	1260
45	225					Arg 230 GAG					235					240	1308
	Ala	Asn	Met	Trp	Pro 245	Glu	Glu	Thr	Leu	Pro 250	Tyr	Phe	Gly	Asp	Gly 255	Asp	
50	GGG Gly	GTC Val	CAC His	ATG Met 260	GCC Ala	TAC Tyr	AAC Asn	TTC Phe	Pro 265	CTG Leu	ATG Met	CCC Pro	CGG Arg	Ile 270	TTC Phe	ATG Met	1356
	GCC Ala	CTA Leu	AGG Arg	CGG	GAG Glu	GAC Asp	CGG Arg	GGT Gly	CCC	ATT Ile	GAA Glu	ACC Thr	ATG Met	CTC	AAG Lys	GAG Glu	1404

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	GCG	GAG	GGG	ATC	CCC	GAA	ACC	GCC	CAG	TGG	GCC	CTC	TTC	CTC	CGC	AAC	1432
	Ala	Glu	Gly	Ile	Pro	Glu	Thr	Ala	Gln	Trp	ATa	Dea	Pne	ren	Arg	ASII	
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	CAC	GAC	GAG	CTC	ACC	CTG	GAG	AAG	GTC	ACG	GAG	GAG	CAG	3	GAG	Pho	1300
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•		TAC	GAG	GCC	TAC	GCC	CCC	GAC	CCC	AAG	TTC	3	TIO	AAC	CTG	Glv	1340
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			355					360	ccc	CAC	220	CCC		CTC	GGG	GAC	1692
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		370					375	» mC	CAG	TGG	TCC	CAA	GAC	CGC	ATC	GTC	1740
20	CGG	AAC	GGT	GTC	AGG	ACC	CCC	Mot	Gla	TTD	Ser	Gln	Asp	Ara	Ile	Val	
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	AAC	Phe	Hig	Ser	Leu	Leu	Ser	Phe	Asn	Arg	Arg	Phe	Leu	Ala	Leu	Arg	
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	CAG	CAA	ccc	TTC	CCC	CCG	GTG	GAG	GGG	7-0	TAC	. CGC	Le	The	CTG	Glv	
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	~=~	GCC	595 CTC	CTG	CAC	GCC	CTC	CGC	TTC	CAG	AAG	GAC	CCG	CCC	CTT	TAC	2412
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						630					033					0 40	2500
		CTC	CTC	TGG	TCC	CCC	CAG	AGG	CGG	GAA	GGC	CCC	GGC	CTC	TTC	GCC	2508
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	720	Ser	Leu	Ara	Ala	TVI	Tyr	Arg	Gly	Arg	His	Pro	Gly	Pro	Val	Pro	
							605					,,,,					2700
	GAG		GTG	GAC	CTC	CTC	CGG	CCG	GGA	CTC	GCG	GCG	GGG	GAG	GGG	GTC	2700
	Glu	GCC Ala	Val	Asp	Leu	Leu	Arg	Pro	Gly	Leu	A10	Ala	GIĀ	GIU	GIY	720	
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		GTC			725	ċmc	CAC	CTC	CCC	TGG	GTT	CTC	CGG	CCC		GGG	2796
	CGG	GTC Val	CTC	DOC	720	Lau	Agn	Leu	Pro	Trp	Val	Leu	Arg	Pro	Glu	Gly	
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	GGA	AGC	CTC	CCC	CCG	GGC	CGC	CCC	CAG	GAC	CTC	TTC	GCC	GUU	CTG	Clu	2052
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							775					700					2940
	GTC	CGG	CTC	CTG	GAA	AGC	CTT	DEC	250	TAU	Ara	เลา	His	Ala	Pro	Gly 800	
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	785		000	CITIC	COM	790	CCC	GCC	CTG	CAC	GAG	ACC	GAA	GCC	CTG	GTC	2988
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45	GTO	GAG	GGG	GTG	GTG	GGG	GGC	CAC	CCC	CTC	CTA	GGC	CGC	GGC	CTC	GGG	3004
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Gln	Ala	Tyr	CGC Arg	Ser	Ala	Leu	920	GAG Glu	GIU	WIG	Deu	925	014	GCG Ala	0-1	3324
Trp	Thr	915 CGG Arg	CAC His	ATG Met	GCC Ala	GAG Glu 935	GTG	GCG Ala	GCG Ala	GAG Glu	CAC His 940	neu	CAC His	CGG Arg	GAG Glu	3372
GAA Glu	930 AGG Arg	CCC Pro	GCC Ala	CGC Arg	Lys	CGC Arg	ATC Ile	CAC His	GAG Glu	CGC Arg 955	TIP	CAG Gln	GCC Ala	AAG Lys	GCC Ala 960	3420
		GCC Ala			950					,,,						3429
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As is described above, the present thermostable enzyme capable of converting maltose into trehalose and *vice versa* which was found as a result of the present inventors' long-term research, and, unlike conventional enzymes, the enzyme has a specific physicochemical properties. The present invention aims to prepare a recombinant enzyme by means of recombinant DNA technology. Referring the following examples, the process for preparing such a recombinant enzyme, its preparation and uses will be described in detail.

The recombinant enzyme as referred to in the present invention includes those in general which are prepared by recombinant DNA technology and capable of converting maltose into trehalose and *vice versa*. Usually the present recombinant DNA has a revealed amino acid sequence, e.g. the amino acid sequence in SEQ ID NO:3 or a homologous amino acid to it. Variants containing amino acid sequences, which are homologous to the amino acid sequence in SEQ ID NO:3, can be prepared by replacing one or more amino acids in SEQ ID NO:3 with other amino acids without alternating the inherent activity of the enzyme. Although even when used the same DNA and it also depends on hosts into which the DNA is introduced, as well as on ingredients and components of nutrient culture media used for culturing transformants, and their cultivation temperature and pH, there may be produced modified enzymes which have the enzymatic activity inherent to the enzyme encoded by the DNA but defect one or more amino acids located in neamess to the N-and/or the C-termini of the amino acid sequence in SEQ ID NO:3, or have one or more amino acids newly added to the N-terminus by the modification of intracellular enzymes of hosts after the DNA expression. Such variants can be included in the present recombinant enzyme as long as they have the desired properties.

The recombinant enzyme according to the present invention can be obtained from cultures of transformants containing the specific DNA. Transformants usable in the present invention can be obtained by introducing into appropriate hosts the base sequence in SEQ ID NO:4, homologous base sequences to it, or complementary base sequences to these base sequences. One or more bases in the above mentioned base sequences may be replaced with other bases by means of the degeneracy of genetic code without alternating the amino acid sequence for which they code. Needless to say, one or more bases in the base sequence, which encodes the enzyme or their variants, can be readily replaced with other bases to allow the DNA to actually express the enzyme production in hosts.

Any DNA derived from natural resources and those artificially synthesized can be used in the present invention as long as they have the aforementioned base sequences. The natural resources of the DNA according to the present invention are, for example, microorganisms of the genus *Thermus aquaticus* (ATCC 33923) from which a gene, containing the DNA used in the present invention, can be obtained. These microorganisms can be inoculated into nutrient culture media and cultured for about 1-3 days under aerobic conditions, and the resultant cells were collected from cultures and subjected to ultrasonication or treated with a cell-wall lysis enzyme such as lysozyme or β-glucanase to extract genes containing the present DNA. In this case, a proteolytic enzyme such as protease can be used in combination with the cell-wall lysis enzyme, and, in the case of treating the cells with ultrasonication, they may be treated in the presence of a surfactant such as sodium dodecyl sulfate (SDS) or treated with the freezing and thawing method. The objective DNA is obtainable by treating the resultant with phenol extraction, alcohol sedimentation, centrifugation, protease treatment and/or ribonuclease treatment used in general in this field. To artificially synthesize the DNA according to the present invention, it can be chemically synthesized by using the base sequence in SEQ ID NO:3, or can be obtained in plasmid form by inserting a DNA, which encodes the amino acid sequence in SEQ ID NO:4, into an appro-

priate self-replicable vector to obtain a recombinant DNA, introducing the recombinant DNA into an appropriate host to obtain a transformant, culturing the transformant, separating the proliferated cells from the resultant culture, and collecting plasmids containing the recombinant DNA from the cells.

Such a recombinant DNA, for example, in the form of a recombinant DNA, is usually introduced into hosts. Generally the recombinant DNA contains the aforesaid DNA and a self-replicable vector and can be prepared by conventional method with a relative easiness when the material DNA is in hand. Examples of such a vector are plasmid vectors such as pBR322, pUC18, Bluescript II SK(+), pKK223-3, pUB110, pTZ4, pC194, pHV14, TRp7, TEp7, pBS7, etc., and phage vectors such as λgt-λC, λgt-λB, ρ11, φ1, φ105, etc. Among these plasmid- and phage-vectors, pBR322, pUC18, Bluescript II SK(+), pKK223-3, λgt-λC and λgt-λB are satisfactorily used in case that the present DNA should be expressed in *Escherichia coli*, while pUB110, pTZ4, pC194, ρ11, φ1 and φ105 are satisfactorily used to express the DNA in microorganisms of the genus *Bacillus*. The plasmid vectors pHV14, TRp7, TEp7 and pBS7 are suitably used when the recombinant DNA is allowed to grow in 2 or more types of hosts.

The methods used to insert the present DNA into such vectors in the present invention may be conventional ones generally used in this field. A gene containing the present DNA and a self-replicable vector are first digested by a restriction enzyme and/or ultrasonic disintegrator, then the resultant DNA fragments and vector fragments are ligated. To ligate DNA fragments and vectors, they may be annealed if necessary, then subjected to the action of a DNA ligase in vivo or in vitro. The recombinant DNA thus obtained is replicable without substantial limitation by introducing it into an appropriate host, and culturing the resultant transformant.

The recombinant DNA according to the present invention can be introduced into appropriate host microorganisms including *Escherichia coli* and those of the genus *Bacillus* as well as actinomyces and yeasts. In the case of using *Escherichia coli* as a host, it can be cultured in the presence of the recombinant DNA and calcium ion, while in the case of using the microorganisms of the genus *Bacillus* the competent cell method and the colony hybridization method can be employed. Desired transformants can be cloned by the colony hybridization method or by culturing a variety of transformants in nutrient culture media containing either maltose or trehalose and selecting transformants which form trehalose or maltose.

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The transformants thus obtained extracellularly produce the objective enzyme when cultured in nutrient culture media. Generally, liquid media in general supplemented with carbon sources, nitrogen sources and/or minerals, and, if necessary, further supplemented with a small amount of amino acids and/or vitamins can be used as the nutrient culture media. Examples of the carbon sources are saccharides such as starch, starch hydrolysate, glucose, fructose and sucrose. Examples of the nitrogen sources are organic- and inorganig-substances containing nitrogen such as ammonia, ammonium salts, urea, nitrate, peptone, yeast extract, defatted soy been, corn steep liquor and beef extract. Cultures containing the objective enzyme can be obtained by inoculating the transformants into nutrient culture media, and incubating them at a temperature of 20-50°C and a pH of 2-9 for about 1-6 days under aerobic conditions by aeration-agitation, etc. Such cultures can be used intact as a crude enzyme preparation, and, usually, cells in the cultures can be disrupted with ultrasonic disintegrator and/or cell-wall lysis enzymes prior to use, followed by separating the enzyme from intact cells and cell debris by filtration and/or centrifugation, and purifying the enzyme. The methods used for purifying the enzyme in the invention include conventional ones in general. From cultures intact cells and cell debris are removed and subjected to one or more methods such as concentration, salting out, dialysis, separately sedimentation, gel filtration chromatography, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and isoelectrophoresis.

As is described above, the present recombinant thermostable enzyme exerts a distinct activity of forming trehalose or maltose from maltose or trehalose respectively even when allowed to act at a temperature of over 55°C, and such an activity has not been found in conventional enzymes. Trehalose has a mild and high-quality sweetness and it has a great advantage of being capable of sweetening food products without fear of causing unsatisfactorily coloration and deterioration because it has no reducing residue within the molecule. By using these properties of the present recombinant thermostable enzyme, maltose, which could not have been used in some field due to its reducibility, can be converted into useful trehalose with a satisfactory handleability and substantial no reducibility.

Explaining now the present enzymatic conversion method in more detail, the wording "maltose" as referred to in the present invention usually means a saccharide composition containing maltose, and any material or method can be used in the present invention as long as trehalose is formed when the present recombinant thermostable enzyme acts thereon or formed thereby. To effectively produce trehalose in an industrial scale, saccharide compositions with a relatively-high maltose content, i.e., usually, about 70 w/w % or more, preferably, about 80 w/w % or more, can be arbitrarily used. Such saccharide compositions can be prepared by conventional methods generally used in this field, for example, those as disclosed in Japanese Patent Publication Nos. 11,437/81 and 17,078/81 wherein β-amylase is allowed to act on gelatinized- or liquefied-starch and separating the formed maltose by separation-sedimentation method or dialysis method, or those as disclosed in Japanese Patent Publication Nos. 13,089/72 and 3,938/79 wherein β-amylase is allowed to act on gelatinized- or liquefied-starch together with a starch debranching enzyme such as isoamylase or pullulanase.

In the enzymatic conversion method according to the present invention, an effective amount of the present recom-

binant thermostable enzyme is allowed to coexist in an aqueous medium containing maltose, followed by keeping the resultant mixture at a prescribed temperature and pH to enzymatically react until the desired amount of trehalose is formed. Although the enzymatic reaction proceeds even at a relatively-low concentration of about 0.1 w/w %, d.s.b., the concentration may be set to about 2 w/w % or more, d.s.b., preferably, about 5-50 w/w %, d.s.b., to proceed the enzymatic conversion method in an industrial scale. The reaction temperature and pH are set within the range which effectively forms maltose without inactivating the recombinant enzyme, i.e. a temperature of over 55°C, preferably, about 56-63°C, and a pH of about 5-10, preferably, about 6-7. The amount of the recombinant enzyme and the reaction time are appropriately set depending on the conditions of the enzymatic reaction. The present enzymatic conversion method effectively converts maltose into trehalose, and the conversion rate reaches up to about 50% or more in some cases.

The reaction mixtures obtainable by the present enzymatic conversion method can be used intact, and, usually, they may be purified prior to use. For example, the reaction mixtures are filtered and centrifuged to remove insoluble substances, and the resultant solutions are decolored with an activated charcoal, desalted and purified with an ion-exchange resin, and concentrated into syrupy products. Depending on use, the syrupy products can be dried *in vacuo* and spray-dried into solid products. To obtain products substantially consisting of trehalose, the syrupy products are subjected to one or more methods of chromatographies using ion exchangers, activated charcoals or silica gels, fermentation using yeasts, and removal by decomposing reducing saccharides with alkalis. To treat a relatively-large amount of reaction mixtures, ion-exchange chromatographies such as fixed bed-, moving bed-, and pseudo-moving bed-methods as disclosed in Japanese Patent Laid-Open Nos.23,799/83 and 72,598/83 are arbitrarily used in the invention, and these enable the effective and large production of high-trehalose content products which have been difficult to obtain in large quantities.

The trehalose and saccharide compositions containing trehalose thus obtained can be used in a variety of products which should be avoided from the reducibility of saccharide sweeteners, and therefore, they can be arbitrarily used in food products in general, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stability, filler, adjuvant or excipient.

The following examples explain the preparation of the recombinant thermostable enzyme and the enzymatic conversion method of maltose according to the present invention:

Example A-1

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Preparation of recombinant enzyme

To 500-ml Erlenmeyer flasks were added 100 ml aliquots of a nutrient culture medium consisting of 2.0 w/v % glucose, 0.5 w/v % peptone, 0.1 w/v % yeast extract, 0.1 w/v % dipotassium hydrogen phosphate, 0.06 w/v % sodium dihydrogen phosphate, 0.05 w/v % magnesium sulfate heptahydrate, 0.5 w/v % calcium carbonate and water, and each flask was sterilized by heating at 115°C for 30 min, cooled, admixed with 50 μg/ml ampicillin, and inoculated with the transformant BTM22 obtained in Experiment 1-2, followed by the incubation at 37°C for 24 hours under rotatory-shaking conditions to obtain a seed culture. To 30-L jar fermenters were added 18 L aliquots of a fresh preparation of the same nutrient culture medium, sterilized similarly as above, admixed with 50 μg/ml ampicillin, and inoculated with 1 v/v % of the seed culture, followed by the incubation at 37°C and a pH of 6-8 for 24 hours under aeration-agitation conditions. The resultant cultures were pooled, treated with ultrasonication to disrupt cells, centrifuged to remove insoluble substances, followed by assaying the enzymatic activity of the resultant supernatant. As a result, one L of the culture contained about 800 units of the recombinant enzyme. The assay of the supernatant conducted by the method in Experiment 1-1 revealed that in this culture was obtained an about 5 ml aqueous solution containing about 152 units/ml of a recombinant enzyme with a specific activity of about 135 units/mg protein.

Example A-2

Preparation of recombinant thermostable enzyme

50 Example A-2(a)

Preparation of transformant BTM23

Recombinant DNA pBTM22, obtained by the method in Example 3-2, was cleaved with *Hind* III, a restriction enzyme, to obtain a DNA fragment consisting of about 8,100 base pairs which contain the base sequence positioning from 107 to 2,889 in SEQ ID NO:4.

Eight oligonucleotides containing base sequences represented by 5'-AGCTTGAATTCTTTTTTAATAAAATCAG-GAGGAAAAACCATGGA CC-3', 5'-CCCTCTGGTACAAGGACGCGGTGATCTACCAGCTCCAC-3', 5'-GTCCGCT CCT-

TCTTTGACGCCAACACGACGCGCTACGG-3', 5'-GGACTTTGAGGGCCTGAGG CGGA-3', 5'-AGCTTCCGCCTCAGGCCCTCAAAGTCCCCGTAGCCGTCGTTGTTG-3', 5'-GCGTCAAAGAAGAAGGAGCGGACGTGGAGCTGGTAGATCACC-3', 5'-GCGTCCTTG TACCAGAGGGGGTCCATGGTTTTTCCTCC-3', and 5'-TGATTTTATTAAAAAAGAA TTCA-3 were mixed in adequate amounts, and the mixture was successively incubated at 100°C 65°C, 37°C and 20°C for 20 min, respectively, to anneal the oligonucleotides. A first recombinant DNA, which contains the base sequence in SEQ ID NO:6 and a base sequence consisting of the bases of positions 1-2,889 in SEQ ID NO:3 wherein the guanines (G) located in the positions 1-963 were replaced with adenines (A), was obtained by adding the above DNA fragment to a double stranded DNA of 141 base pairs having 5' cohesive end of 4 bases at each terminus, which consists of the base sequence in SEQ ID NO:6 and the bases of positions 1-110 in SEQ ID NO:4 wherein the guanine (G) located in the position 1 in SEQ ID NO:4 was replaced with adenine (A) without alternating the amino acid sequence consisting of those of positions 1-36 in SEQ ID NO:3, and allowing the mixture to stand at 4°C overnight in the presence of T4 DNA ligase to anneal the contents.

SEQ ID NO:6:

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AGCTTGAATT CTTTTTTAAT AAAATCAGGA GGAAAAACC

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Recombinant DNA pBTM22 obtained by the method in Experiment 3-2 was cleaved with *Bam* HI, a restriction enzyme, to obtain a DNA fragment consisting of about 2,400 base pairs which contains the base sequence positioning from 1,008 to 2,889 in SEQ ID NO:4 which was then ligated with "M13tv19 RF DNA", a phage vector commercialized by Takara Shuzo Co., Ltd., Tokyo, Japan, which had been cleaved with *Bam* HI to obtain a second recombinant DNA.

An oligonucleotide containing a base sequence represented by 5'-CGGTAGCCCTGCAGCCCCGGG-3' corresponding to the base sequence positioning at 3,438 to 3,458 in SEQ ID NO:5, where "thymine (T)", the base positioning at 3,448 in SEQ ID NO:5 was replaced with "guanine (G)", was in usual manner chemically synthesized. By using the synthesized oligonucleotide and "MUTAN-G", a site-specific mutation system commercialized by Takara Shuzo Co., Ltd., Tokyo, Japan, a third recombinant DNA, which contained the base sequence positioning from 1,008 to 2,889 bases in SEQ ID NO:4 where "thymine (T)", i.e. the base positioning at 3,448 in SEQ ID NO:5, was replaced with "guanine (G)" without alternating the amino acid sequence positioning from 337 to 963 bases in SEQ ID NO:5 which was contained in the second recombinant DNA, was obtained. The procedure of site-specific mutation followed the manual affixed to the "MUTAN-G".

A DNA fragment, consisting of about 1,390 base pairs containing the base sequence positioning at 1 to 1,358 bases in SEQ ID NO:4 where "guanine (G)", i.e. the first base in SEQ ID NO:4, was replaced with "adenine (A)", obtained by cleaving with restriction enzymes *Eco* RI and *BgI* II, and a DNA fragment consisting of abut 1,550 base pairs containing the base sequence positioning at 1,359 to 2,889 in SEQ ID NO:4 obtained by cleaving the third recombinant DNA with restriction enzymes *BgI* II and *Pst* I, were ligated to "pKK223-3", a plasmid vector commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, with T4 DNA ligase to obtain the recombinant DNA pBTM23 containing the base sequence in SEQ ID NO:4.

The recombinant DNA pBTM23 thus obtained was introduced into *Escherichia coli* LE 392 (ATCC 33572) which had been previously prepared into a competent cell according to the method as described by J. Sambrook in "*Molecular Cloning, A Laboratory Manual*", 2nd edition, pp.1.74-1.81 (1989), published by Cold Spring Harbor Laboratory Press, New York, USA, to obtain the present transformant BTM23 containing the DNA coding for the present enzyme. The transformant was cultured by the method in Experiment 3-2, and the proliferated cells were collected from the resultant culture, and lysed to extract the recombinant DNA which was then purified and analyzed, revealing that the recombinant DNA pBTM23 in FIG.6 consisted of about 7,500 base pairs and had a DNA fragment containing 2,889 base pairs which was ligated to the downstream of *Nco* I, a restriction enzyme.

Example A-2(b)

Preparation of recombinant thermostable enzyme using transformant

The transformant BTM23 was cultured similarly as in Example A-1 except that a liquid culture medium (pH 7.0) consisting of one w/v % maltose, 3 w/v % polypeptone, one w/v % "MEAST P1G", a product of Asahi Breweries, Ltd., Tokyo, Japan, 0.1 w/v % sodium dihydrogen phosphate dihydrate, 200 µg/ml ampicillin sodium and water was used. To the resultant culture were added lysozyme from albumen, commercialized by Seikagaku-Kogyo Co., Ltd., Tokyo, Japan, and "TRITON X-100", a surfactant to give respective concentrations of 0.1 mg/ml; and 1 mg/ml, and the resultant was incubated at 37°C for 16 hours while stirring to extract a recombinant thermostable enzyme from the cells. The suspension was heated at 60°C for one hour to inactivate concomitant enzymes from Escherichia coli, followed by centrifuging the mixture to remove impurities, and assaying the enzyme activity in the supernatant, revealing that one L culture contained about 120.000 units of the recombinant thermostable enzyme. The supernatant was purified by the mithod

in Experiment 1 to obtain an about 177 ml aqueous solution containing about 1,400 units/ml of the recombinant thermostable enzyme with a specific activity of about 135 units/mg protein.

The properties and features of the purified enzyme were studied by the method Experiment 2, revealing that it has a molecular weight of 100,000-110,000 daltons on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and an isoelectric point of about 3.8-4.8 on isoelectrophoresis, and it is not inactivated even when incubated at 80°C for 60 min in an aqueous solution (pH 7.0). These physicochemical properties are substantially the same of those of *Thermus aquaticus* (ATCC 33923) as a donor microorganism.

Example B-1

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Preparation of trehalose syrup by recombinant enzyme

Potato starch powder was suspended in water to give a concentration of 10 w/w %, and the suspension was adjusted to pH 5.5, admixed with 2 units/g starch of "SPITASE HS", an α-amylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, and heated at 95°C to effect gelatinization and liquefaction. Thereafter, the resultant liquefied solution was autoclaved at 120°C for 20 min to inactivate the remaining enzyme, promptly cooled to 50°C, adjusted to pH 5.0, admixed with 500 units/g starch of an isoamylase specimen commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, and 20 units/g starch of a β-amylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, and subjected to an enzymatic reaction at 50°C for 24 hours to obtain a saccharide solution containing about 92 w/w % maltose, d.s.b. The saccharide solution was heated at 100°C for 20 min to inactivate the remaining enzyme, cooled to 60 C, adjusted to pH 6.5, admixed with one unit/g starch of the recombinant enzyme obtained in Example A-1, and subjected to an enzymatic reaction for 96 hours. The reaction mixture was heated at 100°C for 10 min to inactivate the remaining enzyme, cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and deionized with an ion-exchange resin, and concentrated to obtain a 70 w/w % syrup in a yield of about 95% to the material starch, d.s.b.

The product contains about 68 w/w % trehalose, d.s.b, and has a relatively-low reducibility because of its DE (dextrose equivalent) 18.4, as well as having a mild sweetness, moderate viscosity and moisture-retaining ability, and these render it arbitrarily useful in a variety of compositions such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, stabilizer, filler, adjuvant or excipient.

Example B-2

Preparation of trehalose powder by recombinant DNA

The reaction mixture obtained in Example B-1 was adjusted to pH 5.0, admixed with 10 units/g starch of "GLU-COZYME", a glucoamylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, and subjected to an enzymatic reaction at 50°C for 24 hours. The reaction mixture thus obtained was heated to inactivate the remaining enzyme, and, in usual manner, decolored, desalted, purified and subjected to ion-exchange column chromatography using "XT-1016 (polymerization degree of 4%)", a cation exchange resin in Na*form commercialized by Tokyo Organic Chemical Industries., Ltd., Tokyo, Japan, to increase the trehalose content. More particularly, the ion-exchange resin, previously suspended in water, was packed in 4 jacketed-stainless steel columns with an inner column diameter of 5.4 cm, and the columns were cascaded in series to give a total column length of 20 m. About 5 v/v % of the reaction mixture was fed to the columns while the inner column temperature was keeping at 60°C, and fractionated by feeding to the columns with 60°C hot water at an SV (space velocity) 0.15, followed by collecting high-trehalose content fractions. The fractions were pooled, and, in usual manner, concentrated, dried in *vacuo*, and pulverized to obtain a trehalose powder in a yield of about 50% to the material, d.s.b.

The product, which contains about 97 w/w % trehalose, d.s.b, and has a relatively-low reducing power and a mild sweetness, can be arbitrarily incorporated into a variety of compositions such as food products, cosmetics and pharmaceuticals as a sweetner, taste-improving agent, stabilizer, filler, adjuvant or excipient.

Example B-3

Preparation of crystalline trehalose powder by recombinant enzyme

A high-trehalose content fraction, obtained by the method in Example B-2, was in usual manner decolored with an activated charcoal, desalted with an ion-exchanger, and concentrated into an about 70 w/w % solution. The concentrate was placed in a crystallizer and gradually cooled while stirring to obtain a massecuite with a crystallization percentage of about 45%. The massecuite was sprayed at a pressure of about 150 kg/cm² from a nozzle equipped at the top of a

drying tower while about 85°C hot air was blowing downward from the top of the drying tower, about 45°C hot air was blowing through under a wire-netting conveyer, which was equipped in the basement of the drying tower, to a crystalline powder collected on the conveyer, and the powder was gradually conveying out from the drying tower. Thereafter, the crystalline powder was transferred to an aging tower and aged for 10 hours in the stream of hot air to complete the crystallization and drying. Thus, a hydrous crystalline trehalose powder was obtained in a yield of about 90% to the material, d.s.b.

The product is substantially free from hygroscopicity and readily handleable, and it can be arbitrarily used in a variety compositions such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stability, filler, adjuvant or excipient.

Example B-4

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Preparation of anhydrous crystalline trehalose powder by recombinant enzyme

A high-trehalose content fraction, obtained by the method in Example B-2, was purified similarly as in Example B-3, and the resultant solution was transferred to a vessel and boiled under a reduced pressure to obtain a syrup with a moisture content of about 3.0 w/w %. The syrup was placed in a crystallizer, admixed with about 1.0 w/w % anhydrous crystalline trehalose as a seed crystal, crystallized at 120°C while stirring, and transferred to a plain aluminum vessel, followed by aging the contents at 100°C for 6 hours to form a block. The block thus obtained was pulverized with a cutter, dried by fluidized bed drying to obtain an anhydrous crystalline trehalose powder with a moisture content of about 0.3 w/w % in a yield of about 85% to the material, d.s.b.

The product with a strong dehydrating activity can be arbitrarily used as a desiccant for food products, cosmetics and pharmaceuticals, as well as their materials and intermediates, and also used as a white powdery sweetener with a mild sweetness in food products, cosmetics and pharmaceuticals.

Example B-5

Preparation of trehalose powder by recombinant enzyme

MALTOSE HHH, a high-purity maltose commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, was dissolved in water to give a concentration of 40 w/w %, heated to 57°C, adjusted to pH 6.5, mixed with 2 units/g maltose, d.s.b., of a recombinant thermostable enzyme obtained by the method in Example A-2, followed by the enzymatic reaction for 48 hours. The reaction mixture was heated at 100°C for 10 min to inactivate the remaining enzyme, cooled, filtered, decolored with an activated charcoal in usual manner, desalted and purified with an ion-exchange resin, dried *in vacuo*, and pulverized to obtain a powdery product containing about 73 w/w % trehalose, d.s.b., in a yield of about 90% to the material maltose, d.s.b.

Although the product has a DE (dextrose equivalent) of 19 which is about 30% of that of maltose, it has the same viscosity as that of maltose, as well as having a mild sweetness and an adequate moisture-retaining ability. Thus, the product can be arbitrarily used as a sweetener, quality-improving agent, stabilizer, filler, adjuvant and excipient in a variety of compositions such as food products, cosmetics and pharmaceuticals.

As is described above, the present invention is based on the finding of a novel thermostable enzyme which forms trehalose or maltose when acts on maltose or trehalose. The present invention aims to explore a way to produce such an enzyme in an industrial scale and in a considerably-high yield by recombinant DNA technology. The enzymatic conversion method using the present recombinant thermostable enzyme converts maltose into a saccharide composition containing trehalose, glucose and/or maltose in a considerably-high yield. Trehalose has a mild and high-quality sweetness, and does not have a reducing residue within the molecule, and because of these it can readily sweeten food products in general without fear of causing unsatisfactory coloration and deterioration. The recombinant enzyme with a revealed amino acid sequence can be used with a greater safety for the preparation of trehalose which is premised to be used in food products.

Therefore, the present invention is a useful invention which exerts the aforesaid significant action and effect as well as giving a great contribution to this field.

While there has been described what is at present considered to be the preferred embodiments of the invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirit and scope of the invention.

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SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- 5 (i) APPLICANT:

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 - (iii) NUMBER OF SEQUENCES:6
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 - (B) COMPUTER: IBM PC compatible
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 - (vii) PRIOR APPLICATION DATA:
 - (A1) APPLICATION NUMBER: JP 260984/94
 - (B1) FILING DATE:October 1, 1994
 - (A2) APPLICATION REFERENCE NUMBER: 10047702
 - (B2) FILING DATE: September 8, 1995
 - (2) INFORMATION FOR SEQ ID NO:1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH:20 amino acids
 - (B) TYPE:amino acid
 - (D) TOPOLOGY:linear
 - (ii) MOLECULE TYPE:peptide
 - (xi) SEQUENCE DESCRIPTION:SEQ ID NO:1:
- Met Asp Pro Leu Trp Tyr Lys Asp Ala Val Ile Tyr Gln Leu His Val
 1 5 10 15
 Arg Ser Phe Phe
 20
 - (3) INFORMATION FOR SEQ ID NO:2:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH:15 amino acids
 - (B) TYPE:amino acid
 - (D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

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(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

Ile Leu Leu Ala Glu Ala Asn Met Trp Pro Glu Glu Thr Leu Pro 1 5 10 15

(4) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH:963 amino acids
 - (B) TYPE:amino acid
 - (D) TOPOLOGY:linear
- (ii) MOLECULE TYPE:peptide
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

	(XI) S	EQUE	ACE DE	SCHIP	TION.S	שבע וט	NO.3.									
20	1		Pro		5					10					15	
20	_		Phe	20					25					30		
		_	Arg 35					40					45			
25	•	50	Met				55					60				
	65		Asp			70					75					80
30			Val		85					90					95	
			Leu	100					105					110		
	_		Pro 115					120					125			-
35		130	Lys				135					140				
	145		Trp			150					155					160
40			Trp		165					170					175	
	_		Ile	180					185					190		
			Arg 195					200					205			
45		210	Glu				215					220				
	225		Leu			230					235					240
50			Met	_	245					250					255	
	_		His	260					265		•			270		
			Arg 275	_				280					285			
55		290	Gly				295					300				
	His	Asp	Glu	Leu	Thr	Leu	Glu	Lys	Val	Thr	Glu	Glu	Glu	Arg	GLu	Phe

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310
                                             315
    305
    Met Tyr Glu Ala Tyr Ala Pro Asp Pro Lys Phe Arg Ile Asn Leu Gly
                                         330
                     325
    Ile Arg Arg Arg Leu Met Pro Leu Leu Gly Gly Asp Arg Arg Tyr
                                     345
                                                         350
                 340
    Glu Leu Leu Thr Ala Leu Leu Leu Thr Leu Lys Gly Thr Pro Ile Val
                                 360
     Tyr Tyr Gly Asp Glu Ile Gly Met Gly Asp Asn Pro Phe Leu Gly Asp
                             375
                                                 380
        370
10
     Arg Asn Gly Val Arg Thr Pro Met Gln Trp Ser Gln Asp Arg Ile Val
                         390
                                             395
     Ala Phe Ser Arg Ala Pro Tyr His Ala Leu Phe Leu Pro Pro Val Ser
                                         410
                                                             415
                     405
     Glu Gly Pro Tyr Ser Tyr His Phe Val Asn Val Glu Ala Gln Arg Glu
                                    425
                 420
     Asn Pro His Ser Leu Leu Ser Phe Asn Arg Arg Phe Leu Ala Leu Arg
                                 440
                                                     445
            435
     Asn Gln His Ala Lys Ile Phe Gly Arg Gly Ser Leu Thr Leu Leu Pro
                                                 460
                             455
     Val Glu Asn Arg Arg Val Leu Ala Tyr Leu Arg Glu His Glu Gly Glu
20
                         470
                                             475
     Arg Val Leu Val Val Ala Asn Leu Ser Arg Tyr Thr Gln Ala Phe Asp
                                         490
                     485
     Leu Pro Leu Glu Ala Tyr Gln Gly Leu Val Pro Val Glu Leu Phe Ser
                                     505
                                                        510
                 500
     Gln Gln Pro Phe Pro Pro Val Glu Gly Arg Tyr Arg Leu Thr Leu Gly
                                                     525
                                 520
     Pro His Gly Phe Ala Leu Phe Ala Leu Lys Pro Val Glu Ala Val Leu
                                                 540
                             535
     His Leu Pro Ser Pro Asp Trp Ala Glu Glu Pro Ala Pro Glu Glu Ala
30
                                             555
                         550
     Asp Leu Pro Arg Val His Met Pro Gly Gly Pro Glu Val Leu Leu Val
                                         570
                     565
     Asp Thr Leu Val His Glu Arg Gly Arg Glu Glu Leu Leu Asn Ala Leu
                                     585
                                                         590
                 580
     Ala Gln Thr Leu Lys Glu Lys Ser Trp Leu Ala Leu Lys Pro Gln Lys
                                 600
     Val Ala Leu Leu Asp Ala Leu Arg Phe Gln Lys Asp Pro Pro Leu Tyr
                                                 620
                             615
     Leu Thr Leu Leu Gln Leu Glu Asn His Arg Thr Leu Gln Val Ser Leu
                                             635
                         630
     Pro Leu Leu Trp Ser Pro Gln Arg Arg Glu Gly Pro Gly Leu Phe Ala
                     645
                                         650
     Arg Thr His Gly Gln Pro Gly Tyr Phe Tyr Glu Leu Ser Leu Asp Pro
                                     665
                 660
45
     Gly Phe Tyr Arg Leu Leu Leu Ala Arg Leu Lys Glu Gly Phe Glu Gly
                                                     685
                                 680
     Arg Ser Leu Arg Ala Tyr Tyr Arg Gly Arg His Pro Gly Pro Val Pro
                             695
                                                 700
     Glu Ala Val Asp Leu Leu Arg Pro Gly Leu Ala Ala Gly Glu Gly Val
                                             715
                         710
     Trp Val Gln Leu Gly Leu Val Gln Asp Gly Gly Leu Asp Arg Thr Glu
                                         730
                     725
     Arg Val Leu Pro Arg Leu Asp Leu Pro Trp Val Leu Arg Pro Glu Gly
                                     745
     Gly Leu Phe Trp Glu Arg Gly Ala Ser Arg Arg Val Leu Ala Leu Thr
```

			755		•			760					765		_	
	_	770					775					780	Ala			
5		Arg				790					795		His			900
·	Thr				$R \cap S$					STO			Glu		010	
10	_			22A	Val				825				Ala	030		
			025	Val				840					Arg 845			
45		050	Leu				855	Glu				800	Ala			
15	~ ~ ~	Lys				חקפ	Glu				8/3		Leu			000
						His				890			Glu		0 9 0	
20	_			$\alpha \alpha \alpha$	Glu				905				Ala	910		
			015	Arg				420					Glu 925			
25		020	Arg				035	Val				940	Leu			
	Glu 945	Arg	Pro	Ala	Arg	Lys 950	Arg	Ile	His	Glu	Arg 955	Trp	Gln	Alą	Lys	Ala 960
	-	Lys	Ala													

(5) INFORMATION FOR SEQ ID NO:4:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH:2889 base pairs (B) TYPE:nucleic acid (D) TOPOLOGY:linear

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

	(···) ·						
40				ATCTACCAGC-	meea cerece	CTCCTTCTTT	60
	GTGGACCCCC		0000		GGCGGAAGCT	TCCCTACCTG	120
	GACGCCAACA	ACGACGGCTA	CGGGGACTTT	GAGGGCCTGA	TCCAGTCCCC	CTTGAGGGAC	180
	GAGGAGCTCG	GGGTCAACAC	CCTCTGGCTC	ATGCCCTTCT		CCTGGAGGAC	240
	GACGGGTACG	ATATCTCCGA	CTACTACCAG	ATCCTCCCCG	TCCACGGGAC	CGTCCTGAAC	300
	TTCACCGTGG	ACGAGGCCCA	CGGCCGGGGG	ATGAAGGTGA	TCATTGAGCT		360
45	CACACCTCCA	TTGACCACCC	TTGGTTCCAG	GAGGCGAGGA	AGCCGAATAG	CCCCATGCGG	420
	GACTGGTACG	TGTGGAGCGA	CACCCCGGAG	AAGTACAAGG	GGGTCCGGGT	CATCTTCAAG	
	GACTTTGAAA	CCTCCAACTG	GACCTTTGAC	CCCGTGGCCA	AGGCCTACTA	CTGGCACCGC	480
	TTCTACTGGC	ACCAGCCCGA	CCTCAACTGG	GACAGCCCCG	AGGTGGAGAA	GGCCATCCAC	540
	CAGGTCATGT	TCTTCTGGGC	CGACCTGGGG	GTGGACGGCT	TCCGCCTGGA	CGCCATCCCC	600
	TACCTCTACG	AGCGGGAGGG	GACCTCCTGC	GAGAACCTCC	CCGAGACCAT	TGAGGCGGTG	660
50	AAGCGCCTGA	GGAAGGCCCT	GGAGGAGCGC		GGAAGATCCT	CCTCGCCGAG	720
	GCCAACATGT	GGCCGGAGGA	GACCCTCCCC		ACGGGGACGG	GGTCCACATG	780
	GCCTACAACT	TCCCCCTGAT	GCCCCGGATC		TAAGGCGGGA	GGACCGGGGT	840
	CCCATTGAAA	CCATGCTCAA	GGAGGCGGAG		AAACCGCCCA	GTGGGCCCTC	900
			GCTCACCCTG		CGGAGGAGGA	GCGGGAGTTC	960
	TTCCTCCGCA	ACCACGACGA	CGACCCCAAG	••••	ACCTGGGGAT	CCGCCGCCGC	1020
55	ATGTACGAGG	CCTACGCCCC			TCCTCACCGC	CCTCCTCCTC	1080
	CTCATGCCCC	TCCTCGGGGG	CGACCGCAGG		TCGGCATGGG	GGACAACCCC	1140
	ACCCTAAAGG	GCACGCCCAT	CGTCTACTAC		GGTCCCAAGA	CCGCATCGTC	1200
	TTCCTCGGGG	ACCGGAACGG	TGTCAGGACC	CCCATGCAGT	GGTCCCHAGA	000000	

	CCCTTCTCCC	GCGCCCCTA	CCACGCCCTC	TTCCTTCCCC	CCGTGAGCGA	GGGGCCCTAC	1260
•	AGCTACCACT	TCGTCAACGT	GGAGGCCCAG	CGGGAAAACC	CCCACTCCCT	CCTGAGCTTC	1320
	AACCGCCGCT	TCCTCGCCCT					1380
5	ACCCTTCTCC	CCGTGGAGAA	CCGCCGCGTC	CTCGCCTACC	TGAGGGAGCA	CGAGGGGGAG	1440
•	CGGGTCCTGG	TGGTGGCCAA		TACACCCAGG		CCCCTTGGAG	1500
	GCCTACCAAG	GCCTCGTCCC			AACCCTTCCC	CCCGGTGGAG	1560
	GGGCGCTACC	GCTTGACCCT			TCTTCGCCCT	GAAGCCCGTG	1620
	GAGGCGGTGC	TCCACCTCCC	CTCCCCGAC	TGGGCCGAGG	AGCCCGCCCC	CGAGGAGGCC	1680
	GACCTGCCCC	GGGTCCACAT	GCCCGGGGGG	CCGGAGGTCC	TCCTGGTGGA	CACCCTGGTC	1740
10	CACGAAAGGG	GGCGGGAGGA	GCTCCTAAAC	GCCCTCGCCC	AGACCCTGAA	GGAGAAGAGC	1800
	TGGCTCGCCC	TCAAGCCGCA		CTCCTGGACG	CCCTCCGCTT	CCAGAAGGAC	1860
	CCGCCCCTTT	ACCTCACCCT	GCTCCAGCTG	GAGAACCACA	GGACCCTCCA	GGTCTCCCTC	1920
	CCCCTCCTCT	GGTCCCCCCA	GAGGCGGGAA	GGCCCCGGCC	TCTTCGCCCG	CACCCACGGC	1980
	CAGCCCGGCT	ACTTCTACGA	GCTCTCCTTG	GACCCAGGCT	TCTACCGCCT	CCTCCTCGCC	2040
15	CGCCTTAAGG	AGGGGTTTGA	GGGGCGGAGC	CTCCGGGCCT	ACTACCGCGG	000000000	2100
	GGTCCCGTGC	CCGAGGCCGT	GGACCTCCTC	CGGCCGGGAC	TCGCGGCGGG	GGAGGGGGTC	2160
	TGGGTCCAGC	TCGGCCTCGT	CCAAGACGGG	GGCCTGGACC	GCACGGAGCG	GGTCCTCCCC	
	CGCCTGGACC	TCCCCTGGGT	TCTCCGGCCC	GAAGGGGGCC	TCTTCTGGGA		2280
	TCCAGAAGGG	TCCTCGCCCT	CACGGGAAGC	CTCCCCCCGG	GCCGCCCCA		2340
20		AGGTCCGGCT	CCTGGAAAGC	CTTCCCCGCC	TCCGGGGGCA	CGCCCCCGGG	2400
20	ACCCCAGGCC	TCCTTCCCGG	GGCCCTGCAC	GAGACCGAAG	CCCTGGTCCG	CCTCCTCGGG	2460
	GTGCGCCTCG	CCCTCCTCCA	CCGGGCCCTT	GGGGAGGTGG	AGGGGGTGGT	GGGGGGCCAC	2520
	CCCCTCCTAG	GCCGCGGCCT	CGGGGCCTTC	CTGGAGCTGG	AGGGGGAGGT	GTACCTCGTG	2580
	GCCCTGGGCG	CGGAAAAGCG	GGGCACGGTG	GAGGAGGACC	TGGCCCGCCT	GGCCTACGAC	2640
	GTGGAGCGGG	CCGTGCACCT	CGCCCTCGAG	GCCCTGGAGG	CGGAGCTTTG	GGCCTTTGCC	2700
25	GAGGAGGTGG	CCGACCACCT	CCACGCCGCC	TTCCTCCAAG	CCTACCGCTC	CGCCCTCCCC	2760
	GAGGAGGCCC	TGGAGGAGGC	GGGCTGGACG		CCGAGGTGGC	GGCGGAGCAC	2820
	CTCCACCGGG	AGGAAAGGCC	CGCCCGCAAG	CGCATCCACG	AGCGCTGGCA	GGCCAAGGCC	2880
•	GGAAAAGCC						2889

(6) INFORMATION FOR SEQ ID NO:5:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH:3600 base pairs
- (B) TYPE:nucleic acid
- (C) STRANDEDNESS:DOUBLE
- (D) TOPOLOGY:linear
- 40 (ii) MOLECULE TYPE:genomic DNA
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Thermus aquaticus
 - (B) INDIVIDUAL ISOLATE: ATCC 33923
 - (ix) FEATURE:

(A)NAME/KEY:5'UTR
(B)LOCATION:1..540

50 (C)IDENTIFICATION METHOD:E
(A)NAME/KEY:mat peptide
(B)LOCATION:541..3429
(C)IDENTIFICATION METHOD:S
(A)NAME/KEY:3'UTR

55 (B)LOCATION:3430..3600
(C)IDENTIFICATION METHOD:E

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

	GCC TGC	CCTC	CCT	CCCC(GGAG	CAACO	CG GG GG CG	CCT	rccc(cttg(GCC	GGGG GTGG	GCC	GCAC. GTGA	AGCC'	rg G	AGGA/ CGGGC	AGGGG	60 12 0
5	CGGG GAGG AAGC	GGGCT	CGG A	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	GAGG GCCT GACA	T GO A GO A GO	AGGC GCCA GCCA	CCGG CGAC AGCG	GCC GCC GCC GCC	TGCC CTCA CTCA	TCG CCG AGGC	AGGC GCAA TCCT CCGC	CTGG GAGG CGGA CTTC	GT T GT C .CG C	'AAGG CTGG CACC GCCT	ACTAC CGGTG AGCTC CGGAC TAAGG	180 240 300 360 420 480
40	GGCC	GGGG	CC I	CACC	TTCA	A GO	7.666	100A	1 GGC	いてなかい	ייירר	GACC	COLL	אה כ	CTTT	TCAAG	540
10	GACG	TGG	AGG P	GGTC	,C166	E 2 C 1	VVCI I	CAC	CCC	CTG	חדת	TAC	CAG	CTC	CAC	GTC	588
	Met	Asp	Pro	Leu	Trp	Tyr	Lys	Asp	Ala	Val 10	Ile	Tyr	Gin	Leu	15	vaı	
	CCC	TCC	TTC	TTT	GAC	GCC	AAC	AAC	GAC	GGC	TAC	GGG	GAC	TTT	GAG	GGC	636
15	Arg	Ser	Phe	Phe	Asp	Ala	Asn	Asn	Asp	Gly	Tyr	Gly	Asp	Phe	Glu	Gly	
,,,	_			20					25					30			604
	CTG	AGG	CGG	AAG	CTT	CCC	TAC	CTG	GAG	GAG	CTC	GGG	GTC	AAC	ACC	CTC	684
		_	25					40					45		Thr		732
20	TGG	CTC	ATG	CCC	TTC	TTC	CAG	TCC	CCC	TTG	AGG	ACD	ACD	Glv	TAC	Asn	, 52
20	Trp		Met	Pro	Phe	Pne	55	Ser	PIO	Leu	AL Y	60 60	vaħ	GLY	Tyr	nop	
	N THE	50 TCC	CAC	TAC	TAC	CAG	ATC	CTC	CCC	GTC	CAC	GGG	ACC	CTG	GAG	GAC	780
	Tle	Ser	Asp	TVI	Tvr	Gln	Ile	Leu	Pro	Val	His	Gly	Thr	Leu	Glu	Asp	
	6 5					70					75					80	
25	ጥጥር	ACC	GTG	GAC	GAG	GCC	CAC	GGC	CGG	GGG	ATG	AAG	GTG	ATC	ATT	GAG	828
	Phe	Thr	Val	Asp	Glu	Ala	His	Gly	Arg	Gly	Met	Lys	Val	Ile	lle 95	Glu	
	0.00	C.W.C.	CEC	አአሮ	85 CAC	NCC.	TrCC	Δ·ψ·ψ·	GAC	90 CAC	ССТ	TĠG	TTC	CAG	GAG	GCG	876
	CTC	Ual	Leu	AAC	His	Thr	Ser	Ile	Asp	His	Pro	Trp	Phe	Gln	Glu	Ala	
				100					105					TIO			
30	AGG	AAG	CCG	ידית מ	AGC	CCC	ATG	CGG	GAC	TGG	TAC	GTG	TGG	AGC	GAC	ACC	924
	Arg	Lys	Pro	Asn	Ser	Pro	Met	Arg	Asp	Trp	Tyr	Val	125	Ser	Asp	TOY	0.50
	CCG	GAG	AAG	TAC	AAG	GGG	GTC	CGG	GTC	ATC	TTC	AAG	GAC	TTT	GAA	ACC	972
	Pro	Glu	Lys	Tyr	Lys	GŢĀ	Val 135	Arg	Val	Ile	Phe	Lys 140	Asp	Phe	GIU	Thr	
35	TICC.	130	TCC	ACC	արարար	GÁC	CCC	GTG	GCC	AAG	GCC	TAC	TAC	TGG	CAC	CGC	1020
	Ser	Asn	Trp	Thr	Phe	Asp	Pro	Val	Ala	Lys	Ala	Tyr	Tyr	Trp	His	Arg	
	145					150					155					100	
	TTC	TAC	TGG	CAC	CAG	CCC	GAC	CTC	AAC	TGG	GAC	AGC	CCC	GAG	GTG	GAG	1068
40	Phe	Tyr	Trp	His	Gln	Pro	Asp	Leu	Asn	Trp	Asp	Ser	Pro	GIU	175	GIU	1116
	AAG	GCC	ATC	CAC	CAG	GTC	ATG	TTC	TTC	TGG	GCC	BAC	Lau	Glv	GTG	Asn	1110
	Lys	Ala	Ile		Gin	vaı	мет	Pne	185	TIP	VIG	vəħ	Deu	190	Val		
	ccc	mmc	ccc	180	GAC	GCC	ATC	CCC	TAC	CTC	TAC	GAG	CGG		GGG	ACC	1164
	GUU	Phe	Arg	T.eu	ASD	Ala	Ile	Pro	Tyr	Leu	Tyr	Glu	Arg	Glu	Gly	Thr	
45			195					200					205				
	TCC	TGC	GAG	AAC	CTC	CCC	GAG	ACC	ATT	GAG	GCG	GTG	AAG	CGC	CTG	AGG	1212
	Ser	Cys	Glu	Asn	Leu	Pro	Glu	Thr	Ile	Glu	Ala	vaı	rys	Arg	Leu	Arg	
		210					215					ZZU					1260
50	AAG	GCC	CTG	GAG	GAG	CGC	TAC	01	D	GGG	Lare	TIA	TAI	Len	GCC Ala	Glu	
50	Lys 225	ATB	ьeu	GIU	GIU	230		GIĀ	FIO	Gry	235					240	
	GCC	AAC	ATG	TGG	CCG	GAG	GAG	ACC	CTC	CCC	TAC	TTC	GGG	GAC	GGG	GAC	1308
	Ala	Asn	Met	Trp	Pro	Glu	Glu	Thr	Leu	Pro	Tyr	Phe	Gly	Asp	GIA	Asp	
					245					250					455		1056
55	GGG	GTC	CAC	ATG	GCC	TAC	AAC	TTC	CCC	CTG	ATG	CCC	CGG	ATC	TTC	ATG	1356

	Gly	Val	His	Met 260	λla	Tyr	Asn	Phe	Pro 265	Leu	Met	Pro	Arg	Ile 270	Phe	Met	
	000	Cm a	N.C.C	CGG	GAG	CAC	CGG	GGT	CCC	ATT	GAA	ACC	ATG		AAG	GAG	1404
5	Ala	Leu	Arg	Arg	Glu	Asp	Arg	Gly 280	Pro	Ile	Glu	Thr	Met 285	Leu	Lys	Glu	
	CCC	GAG	CCC	ATC	CCC	GAA	ACC	GCC	CAG	TGG	GCC	CTC	TTC	CTC	CGC	AAC	1452
	Ala	Glu	Gly	Ile	Pro	Glu	Thr	Ala	Gln	Trp	Ala	Leu 300	Phe	Leu	Arg	Asn	
		290				CMC	295	* * C	CTC	ACG	CAG	GAG	GAG	ccc	GAG	ጥጥር	1500
10	CAC	GAC	GAG	CTC	ACC	LTG	GAG	LAC	Val	Thr	Glu	Glu	Glu	Arg	Glu	Phe	2000
		Asp	Glu	Leu	Thr	310	GIU	LYS	VGI	1111	315	014	014	9	0-0	320	
	305	mac.	CAC	GCC	ሞልሮ	GCC	CCC	GAC	CCC	AAG	TTC	CGC	ATC	AAC	CTG	GGG	1548
	Mot	TAC	Clu	112	TUT	Ala	Pro	Asp	Pro	Lvs	Phe	Arg	Ile	Asn	Leu	Gly	
		-			325					330					33 3		
15	ATC	CGC	CGC	CGC	CTC	ATG	CCC	CTC	CTC	GGG	GGC	GAC	CGC	AGG	CGG	TAC	1596
	Ile	Arg	Arg	Arg 340	Leu	Met	Pro	Leu	Leu 345	Gly	Gly	yab	Arg	Arg 350	Arg	Tyr	
	GAG	רתר	CTC	ACC	GCC	CTC	CTC	CTC	ACC	CTA	AAG	GGC	ACG	CCC	ATC	GTC	1644
	Glu	Leu	Leu	Thr	Ala	Leu	Leu	Leu	Thr	Leu	Lys	Gly	Thr	Pro	Ile	Val	
			255					360					365				
20	TAC	TAC	CCC	GAC	GAG	ATC	GGC	ATG	GGG	GAC.	AAC	CCC	TTC	CTC	GGG	GAC	1692
	Tyr	Tyr	Gly	Asp	Glu	Ile	Gly 375	Met	Gly	Asp	Asn	Pro 380	Phe	Leu	Gly	Asp	
		370	CCT	GTC	AGG.	ACC	CCC	ATG	CAG	TGG	TCC	CAA	GAC	CGC	ATC	GTC	1740
	V.C.C.	AAC	Glv	Val	Arg	Thr	Pro	Met	Gln	Trp	Ser	Gln	Asp	Arg	Ile	Val	
25	205					390					395					400	
	CCC	TTC	TCC	CGC	GCC	CCC	TAC	CAC	GCC	CTC	TTC	CTT	CCC	CCC	GTG	AGC	1788
	Ala	Phe	Ser	Arg	Ala 405	Pro	Tyr	His	Ala	Leu 410	Phe	Leu	Pro	Pro	Val 415	Ser	
	CAC	CCC	CCC	ጥልር	405 ACC	ጥልሮ	CAC	TTC	GTC	AAC	GTG	GAG	GCC	CAG	CGG	GAA	1836
	GAG	GGG	Pro	TAC	Ser	Tvr	His	Phe	Val	Asn	Val	Glu	Ala	Gln	Arg	Glu	
30				420					425					430			1884
	AAC	CCC	CAC	TCC	CTC	CTG	AGC	TTC	AAC	CGC	720	Pho	Leu	Ala	CTG	Ara	1004
			125					440					445		Leu		1000
	AAC	CAG	CAC	GCC	AAG	ATC	TTC	GGC	CGG	GGG	AGC	CTC	ACC	CTT	CTC	Doo	1932
35		450					455					400			Leu		
	GTG	GAG	AAC	CGG	CGC	GTC	CTC	GCC	TAC	CTG	AGG	GAG	CAC	GAG	GGG	GAG	1980
	Val	Glu	Asn	Arg	Arg	Val	Leu	Ala	TYI	Leu	Arg	Glu	His	Glu	Gly	GLU	
	465					470					475					400	2020
40	CGG	GTC	CTG	GTG	GTG	GCC	AAC	CTC	TCC	CGC	TAC	ACC	CAG	GCC	TTT	GAC	2028
					485					490	-				Phe 495		
	CTC	CCC	TTG	GAG	GCC	TAC	CAA	GGC	CTC	GTC	CCC	GTG	GAG	CTC	TTC	TCG	2076
	Leu	Pro	Leu	Glu	Ala	Tyr	Gln	Gly	Leu 505	Val	Pro	Val	Glu	Leu 510	Pne	Ser	
45	a. a	~~~	~~~	500	ccc	ccc	CEC	CAG	GGG	CGC	TAC	CGC	TTG		CTG	GGC	2124
45	CAG	CAA	BEO	Pho	Pro	Pro	Val	Glu	GOG	Arg	Tvr	Arg	Leu	Thr	Leu	Gly	
			515					520					525				0170
	CCC	CAC	GGC	TTC	GCC	CTC	TTC	GCC	CTG	AAG	ccc	GTG	GAG	GCG	GTG	CTC	2172
	Pro			Phe	Ala	Leu	Phe	Ala	Leu	Lys	Pro	Val	GIU	AT8	var	Leu	
50		530				a. c	535	~~~	C1.C	~~	000	540		GAC	CVC	GCC	2220
	CAC	CTC	CCC	TCC	CCC	GAC	166	31-	GAG	Clin	D-C	- [α	Pro	Glu	GAG Glu	Ala	
	H1S 545		Pro	ser	PIO	550		WIG	GLU	GIU	555	770		<u></u>		560	
	543					550											

	GAC	CTG	CCC	CGG	GTC	CAC	ATG	CCC	GGG	GGG	CCG	GAG	GTC	CTC	CTG	GTG	2268
	Asp	Leu	Pro	Arg	Val 565	His	Met	Pro	Gly	Gly 570	Pro	GLu	Val	Leu	Leu 575	Val	
	GAC	ACC	CTG	GTC	CAC	GAA	AGG	GGG	CGG	GAG	GAG	CTC	CTA	AAC	GCC	CTC	2316
5	- Asp	Thr	Leu	Vla	His	Glu	Arg	Gly	Arg 585	Glu	Glu	Leu	Leu	Asn 590	Ala	Leu	
	GCC	CAG	ACC	CTG	AAG	GAG	AAG	AGC	TGG	CTC	GCC	CTC	AAG	CCG	CAG	AAG	2364
	Ala	Gln	Thr	Leu	Lys	Glu	Lys	Ser 600	Trp	Leu	Ala	Leu	Lys 605	PTO	GIN	rÅa	
10	GTG	GCC	CTC	CTG	GAC	GCC	CTC	CGC	TTC	CAG	AAG	GAC	CCG	CCC	CTT	TAC	2412
	Val	Ala.	Leu	Leu	Asp	Ala	Leu 615	Arg	Phe	Gln	Lys	Asp 620	Pro	PTO	гÃг	TYL	2460
	CTC	ACC.	CTG	CTC	CAG	CTG	GAG	AAC	CAC	AGG	ACC	CTC	CAG	GTC	TCC	CTC	2460
	Leu	Thr	Leu	Leu	Gln	Leu	Glu	Asn	His	Arg	Thr	Leu	Gin	Val	Ser	Leu	
15	625					หรอ					635					040	2508
	CCC	CTC	CTC	TGG	TCC	CCC	CAG	AGG	CGG	GAA	GGC	CCC	Clar	Tou	Pho	Ala	2300
		Leu			615					650					000		2556
	CGC	ACC	CAC	GGC	CAG	CCC	GGC	TAC	TTC	TAC	Clu	Lau	Sor	Len	Acn	Pro	2550
20	-	Thr		660					665					0/0			0.004
	GGC	TTC	TAC	CGC	CTC	CTC	CTC	GCC	CGC	CTT	AAG	GAG	GGG	TTT	GAG	GGG	2604
	Gly	Phe	Tyr	Arg	Leu	Leu	Leu	Ala 680	Arg	Leu	Lys	GIU	685	Pne	GIU	GIY	
	CGG	AGC	CTC	CGG	GCC	TAC	TAC	CGC	GGC	CGC	CAC	CCG	GGT	CCC	GTG	CCC	2652
25	Arg	Ser	Leu	Arg	Ala	Tyr	Tyr	Arg	Gly	Arg	His	Pro 700	Gly	Pro	·Val	Pro	
		690 GCC	a=0	~~~	amc	CTC	695	CCG	CCA	CTC	CCG		GGG	GAG	GGG	GTC	2700
	GAG	Ala	GTG	ACC	Lov	Lou	Arg	PTO	Glv	Leu	Ala	Ala	Glv	Glu	Gly	Val	
,	705					710					\T2					120	
	TCC	GTC	CAG	CTC	GGC	CTC	GTC	CAA	GAC	GGG	GGC	CTG	GAC	CGC	ACG	GAG	2748
30	Tro	Val	Gln	Leu	Gly	Leu	Val	Gln	Asp	Gly	Gly	Leu	Asp	Arg	inr	Glu	
					725					730					/35		2706
	CGG	GTC	CTC	CCC	CGC	CTG	GAC	CTC	CCC	TGG	GTT	CTC	CGG	CCC	GAA	Class	2796
		Val		740					745					750			
	GGC	CTC	TTC	TCC	GAG	CGG	GGC	GCC	TCC	AGA	AGG	GTC	CTC	GCC	CTC	ACG	2844
35	Gly	Leu	Phe	Trp	Glu	Arg	Gly	Ala	Ser	Arg	Arg	Val	Leu 765	Ala	Leu	Thr	
		AGC	755			ccc	ccc	760	CAG	GAC	CTC	TTC		GCC	CTG	GAG	2892
	GGA Gly	Ser	Leu	Pro	Pro	Gly	Arg	Pro	Gln	Asp	Leu	Phe	Ala	Ala	Leu	Glu	
	_	770					775					780					2940
40	GTC	CGG	CTC	CTG	GAA	AGC	CTT	ccc	CGC	CTC	CGG	GGG	CAU	310	Bro	Clv	2940
	725	Arg				790					795					800	2000
	ACC	CCA	GGC	CTC	CTT	CCC	GGG	GCC	CTG	CAC	GAG	ACC	GAA	GCC	CTG	Unl	2988
		Pro			805					810					913		
45	CGC	CTC	CTC	GGG	GTG	CGC	CTC	GCC	CTC	CTC	CAC	CGG	GCC	CTT	GGG	GAG	3036
	Arg	Leu	Leu	Gly 820	Val	Arg	Leu	Ala	Leu 825	Leu	His	Arg	Ala	830	GIY	GIU	
	GTG	GAG	GGG	GTG	GTG	GGG	GGC	CAC	CCC	CTC	CTA	GGC	CGC	GGC	CTC	GGG	3084
50	Val	Glu	Gly	Val	Val	Gly	Gly	His 840	Pro	Leu	Leu	Gly	845	GTĀ	Leu	GIY	
	GCC	TTC	CTG	CAG	CTG	GAG	GGG	GAG	GTG	TAC	CTC	GTG	GCC	CTG	GGC	GCG	3132
	Ala	Phe	Leu	Glu	Leu	Glu	Gly	Glu	Val	Туг	. Leu	Val	Ala	Leu	Gly	Ala	

	GAA Glu	850 AAG Lys	CGG Arg	GGC GGC	TIT						875				-	000	3180
5	865 GTG Val	GAG Glu	CGG Arg	GCC Ala	Val	CAC His	Leu	Ala	Leu	890	ATG	Dea	GAG Glu	••	GAG Glu 895 TTC	Leu	3276
10				Ala	GAG Glu	Glu	Val	ATS	905	UIS		cmc	Ala	910 GAG	Phe	Leu GGC	3324
	CAA Gln	GCC Ala	Tyr		Ser	710		Pro 920	Glu	Glu	Ale	Leu	925 270			GAG	3372
15	Trp	000	Arg	His	Met	AIB	935	Val	7,10			940	C	GCC	AAG	GCC Ala	3420
	G1:	Arg	Pro) Als	Arg	Lys 950	y	Ile	His	s Glu	95	g Trp	GIT	I ATC	, ny.	960	3429
20	Gly	rece	A GCC s Ala 960 CCCG	a 3 GTA(30001 10001	- CM 4	'C'T'T'(CCCC	CG GC	JGG 17	3GAC (9 60	GGGT GTCCC			CGGCCTC GGCAGA#	3489 3549 3600
25	GG GG	CGGG CGCA	GAGG CACC	AGG(CCCG'		rGGG	GTAG(CC G	CACC	GCTC	G CA	CTCC	JIAA			

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH:39 base pairs
- (B) TYPE:nucleic acid
- (D) TOPOLOGY:linear
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

AGCTTGAATT CTTTTTTAAT AAAATCAGGA GGAAAAACC

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Claims 40

- 1. A recombinant enzyme which is capable of converting maltose into trehalose and not substantially inactivated even when incubated at a temperature of over 55°C.
- 2. The recombinant enzyme of claim 1, which has the following physicochemical properties: 45

Forming trehalose when acts on maltose, and vice versa;

About 100,000-110,000 daltons when assayed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (2) Molecular weight (MW) (SDS-PAGE);

(3) Isoelectric point (pl)

About 3.8-4.8 when assayed on isoelectrophoresis;

(4) Optimum temperature

About 65°C when incubated at pH 7.0 for 60 min;

(5) Optimum pH

About 6.0-6.7 when incubated at 60 C for 60 min;

(6) Thermal stability

Stable up to a temperature of about 80°C even when incubated at pH 7.0 for 60 min; and

Stable up to a pH of 5.5-9.5 even when incubated at 60°C for 60 min.

The recombinant enzyme of claim 1, which has the amino acid sequences of SEQ ID NOs:1 and 2 as a partial amino acid sequence:

SEQ ID NO:1:

Met Asp Pro Leu Trp Tyr Lys Asp Ala Val Ile Tyr Gln Leu His Val 10 Arg Ser Phe Phe 20

SEQ ID NO:2: 15

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Ile Leu Leu Ala Glu Ala Asn Met Trp Pro Glu Glu Thr Leu Pro 1

The recombinant enzyme of claim 1, which has an amino acid sequence selected from the group consisting of the amino acid sequence in SEQ ID NO:3, and homologous amino acid sequences thereunto:

SEQ ID NO:3: Met Asp Pro Leu Trp Tyr Lys Asp Ala Val Ile Tyr Gln Leu His Val 25 Arg Ser Phe Phe Asp Ala Asn Asn Asp Gly Tyr Gly Asp Phe Glu Gly Leu Arg Arg Lys Leu Pro Tyr Leu Glu Glu Leu Gly Val Asn Thr Leu Trp Leu Met Pro Phe Phe Gln Ser Pro Leu Arg Asp Asp Gly Tyr Asp 30 Ile Ser Asp Tyr Tyr Gln Ile Leu Pro Val His Gly Thr Leu Glu Asp Phe Thr Val Asp Glu Ala His Gly Arg Gly Met Lys Val Ile Ile Glu 35 Leu Val Leu Asn His Thr Ser Ile Asp His Pro Trp Phe Gln Glu Ala Arg Lys Pro Asn Ser Pro Met Arg Asp Trp Tyr Val Trp Ser Asp Thr Pro Glu Lys Tyr Lys Gly Val Arg Val Ile Phe Lys Asp Phe Glu Thr 40 Ser Asn Trp Thr Phe Asp Pro Val Ala Lys Ala Tyr Tyr Trp His Arg Phe Tyr Trp His Gln Pro Asp Leu Asn Trp Asp Ser Pro Glu Val Glu

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Lys Ala Ile His Gln Val Met Phe Phe Trp Ala Asp Leu Gly Val Asp

	100
	180 185 180 185 Gly Phe Arg Leu Asp Ala Ile Pro Tyr Leu Tyr Glu Arg Glu Gly Thr 205 200 200 200 200 200 200 200 200 200
	180 Asp Ala Ile Pro Tyr Leu Tyr Glu 1125
5	Ser Cys Glu Asn Leu Pro Glu Till 120 220 215 210 210 240 240 230 230 230 255 257
	710 - Maria CIV PIO 047 -2" 240
	Lys Ala Leu Glu 230 230 Pro Tyr Phe Gly Asp Gly Asp
	Lys Ala Leu Glu Glu Arg Tyl Gly 235 230 225 Ala Asn Met Trp Pro Glu Glu Thr Leu Pro Tyr Phe Gly Asp Gly Asp 255 Ala Asn Met Trp Pro Glu Glu Thr Leu Pro Tyr Phe Gly Asp 255 Ala Asn Met Trp Pro Glu Glu Thr Leu Pro Arg Ile Phe Met
10	
	Ala Asn Met Trp Pro Glu Glu Thr Leu 250 Ala Asn Met Trp Pro Glu Glu Thr Leu 250 245 Gly Val His Met Ala Tyr Asn Phe Pro Leu Met Pro Arg Ile Phe Met 270 265 Cly Pro Ile Glu Thr Met Leu Lys Glu
15	Ala Glu Gly Ile Pro Glu Thr Ala 300 295 290 His Asp Glu Leu Thr Leu Glu Lys Val Thr Glu Glu Glu Arg Glu Phe 310 320 320 310 310 310 325
	His Asp Glu Leu Thr Leu Glu Lys Val 315 310 305 Met Tyr Glu Ala Tyr Ala Pro Asp Pro Lys Phe Arg Ile Asn Leu Gly 335 Met Tyr Glu Ala Tyr Ala Pro Asp Pro Lys Phe Arg Ile Asn Leu Gly 335 Met Tyr Glu Ala Tyr Ala Pro Asp Pro Lys Phe Arg Arg Arg Tyr
20	
	Glu Leu Leu Thr Ala Leu 360 360 Pro Phe Leu Gly Asp
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25	
	Tyr Tyr Gly Asp Glu Ile Gly Met Gln Trp Ser Gln Asp Arg Ile Val 375 400 Arg Asn Gly Val Arg Thr Pro Met Gln Trp Ser Gln Asp Arg Ile Val 395 385 Ala Phe Ser Arg Ala Pro Tyr His Ala Leu Phe Leu Pro Pro Val Ser 410 405 410 407
30	Glu Gly Pro Tyr Ser Tyr Arg Phe Leu Ala Leu Arg
	433 - SLA CIU AIU GAI
35	Asn Gln His Ala Lys 110 455 455 450 480
35	
	Arg Val Leu Val Val Ala Ash Leu Set 490 485 Leu Pro Leu Glu Ala Tyr Gln Gly Leu Val Pro Val Glu Leu Phe Ser 505 Leu Pro Leu Glu Ala Tyr Gln Gly Arg Tyr Arg Leu Thr Leu Gly
40	
	tal CIN GIV Pry
	Gln Gln Pro Phe Pro Pro Val Glu Ala Val Leu 515 Pro His Gly Phe Ala Leu Phe Ala Leu Lys Pro Ala Pro Glu Glu Ala 520 520 540 535
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	His Leu Pro Ser Plo Asp 550 550 Glu Val Leu Leu Val
	San San Gly Alu Gly VIII EQU
5	Asp Thr Leu Val His Glu Arg 517 585 580 580 605 600 Ala Gln Thr Leu Lys Glu Lys Ser Trp Leu Ala Leu Lys Pro Gln Lys
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	Val Ala Leu Leu Asp Ala 615 Thr Leu Gln Val Ser Leu
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	635 630 630 655 650 655 650 645 655 650 650 650 650 650 650 650 670
٠,	630 Gln Arg Arg Glu Gly Plo Gli 655
D-	ro Leu Leu Trp Ser Pro Gln Arg Arg G50 ro Leu Leu Trp Ser Pro Gln Arg Arg G50 645 rg Thr His Gly Gln Pro Gly Tyr Phe Tyr Glu Leu Ser Leu Asp Pro 665 rg Thr His Gly Gln Pro Gly Tyr Phe Tyr Glu Leu Ser Leu Asp Pro 665 660 rg Thr His Gly Gln Pro Gly Ala Arg Leu Lys Glu Gly Phe Glu Gly 665
F -	670 Cln Pro Gly Tyr Phe Tyr Glu Gly Gly
⁵ A	rg Thr His Gly Glu Gly Phe Gly
	rg Thr His Gly Gln Pro Gly Tyr Phe 192 670 665 660 680 680 675 680 680 680 680 680 680 680 680 680 680
G	Sly Phe Tyr Arg Leu Leu Leu Ala Arg Leu 27 685 680 680 675 675 700 675 700 695 690 695 690 720
,	Arg Ser Leu Arg Ala Tyr Tyl Mag 700 Arg Ser Leu Ala Gly Glu Gly Val 720
10	Arg Ser Leu Arg Ala Tyr Tyr Arg Gly Ala Tov 700 690 690 Glu Ala Val Asp Leu Leu Arg Pro Gly Leu Ala Ala Gly Glu Gly Val 720 715
(Glu Ala Val ASP Leu 200 710
	705 - 725
	Trp Val Gln Leu Gly Leu Val Gln Asp 730 725 Arg Val Leu Pro Arg Leu Asp Leu Pro Trp Val Leu Arg Pro Glu Gly 750 745 740 740 745 745 746
15	725 Tou Asp Leu Pro Trp Val Leu Arg 750
	Arg Val Leu Pro Arg Leu Asp Leu Pro 745 745 740 Gly Leu Phe Trp Glu Arg Gly Ala Ser Arg Arg Val Leu Ala Leu Thr 765 760 7757 7757 7757 7757 7757 7757 7
•	740 Arg Gly Ala Ser Arg Rig 765
	Gly Leu Phe Trp Glu Arg Gly Ala Ser Arg 765 760 755 Gly Ser Leu Pro Pro Gly Arg Pro Gln Asp Leu Phe Ala Ala Leu Glu 7780 780 780 780 780 780 780 780 780 78
20	755 Pro Pro Gly Arg Pro Gin 780 780 780 780 780 780 780 780 780 780
	Gly Ser Leu Pro Pro Gly Arg Pro Gli Asp 780 775 770 770 Val Arg Leu Leu Glu Ser Leu Pro Arg Leu Arg Gly His Ala Pro Gly 790 Val Arg Leu Leu Glu Ser Leu Pro Arg Leu His Glu Thr Glu Ala Leu Val 790 815
	Val Arg Leu Leu Glu Ser Leu 11 795 Thr Glu Ala Leu Val
	Val Arg Leu Leu Glu Ser Leu Pro Arg Leu 795 Val Arg Leu Leu Glu Ser Leu Pro Arg Leu 795 785 Thr Pro Gly Leu Leu Pro Gly Ala Leu His Glu Thr Glu Ala Leu Val 815 Thr Pro Gly Leu Leu Pro Gly Ala Leu His Arg Ala Leu Gly Glu 805
25	785 Thr Pro Gly Leu Leu Pro Gly Ala Leu Ris 810 810 830 Arg Leu Leu Gly Val Arg Leu Ala Leu Leu His Arg Ala Leu Gly Glu 820 825 820 826 827 827 828
25	Low Leu Gly Val Arg Leu Ala Bea 25 Str. Arg Gly Leu Gly
	Arg Leu Bou 820 845
	Arg Leu Leu Gly Val Arg Leu Ala Leu Leu Gly Arg Gly Leu Gly Val Glu Gly Val Val Gly Gly His Pro Leu Leu Gly Arg Gly Leu Gly 845 840 835 Ala Phe Leu Glu Leu Glu Gly Gly Val Tyr Leu Val Ala Leu Gly Ala 855 855 850 860 880
	835 Gly Leu Glu Gly Glu Val Tyr Bed 860
30	Ala Phe Leu Glu Leu Glu Gly Glu Val 172 860 855 850 Glu Lys Arg Gly Thr Val Glu Glu Asp Leu Ala Arg Leu Ala Tyr Asp 875 870 870 895
	850 Arg Gly Thr Val Glu Glu RSP 875
	Glu Lys Ala Leu Glu Ala Leu Glu Ala Leu Glu 895
	Glu Lys Arg Gly Thr Val Glu Glu Asp Leu 875 870 865 Val Glu Arg Ala Val His Leu Ala Leu Glu Ala Leu Glu Ala Clu Leu 895 Trp Ala Phe Ala Glu Glu Val Ala Asp His Leu His Ala Ala Phe Leu 900 905 900 900 905 907 908
35	Glu Glu Val Ala Asp HIS Book 910
	Trp Ala Phe Ala Car 900
	Trp Ala Phe Ala Glu Glu Val Ala Asp nis 910 905 900 Gln Ala Tyr Arg Ser Ala Leu Pro Glu Glu Ala Leu Glu Glu Ala Gly 925 920 920 915 920 920 920 920 920 920 920 920 920 920
	915 Sin Ala Ala Glu His Lea
40	Gln Ala Tyr Arg Ser Ala Leu Pro Glu
	930 Na Arg Lys Arg Ile His Giu Als 955
•	Glu Arg Pro Ald 950
	945 Gly Lys Ala
45	GIX DI-

5. A DNA which encodes the recombinant enzyme of claim 1.

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 The DNA of claim 5, which has a base sequence selected from thr group consisting of the base sequence in SEQ ID NO:4, homologous base sequences thereunto, and complementary base sequences to these base sequences. 50

	SEQ ID NO:4: GTGGACCCCC TCTGGTACAA GGACGCGGTG ATCTACCAGC TCCACGTCCG CTCCTTCTT TCCACGTCCC TCCCTACCTG 120 120 120 120 120 120 120 120
	GTGGACCCC TCTGGTACAA GGACGCGGTG ATCTACCTGA GGCGGAAGCT TCCCTGAGGGAC CTTGAGGGAC CTTGAGGGAC CTTGAGGGAC CTTGAGGGAC CCTGGGAGACAC CCTCTGGCTC ATGCCCTTCT TCCACGGGAC CCTGGAGGAC CCTGGAGACAC CCTCTGACCAC ATCCTCCCCG TCCACGGGAC CGTCCTGAAC 300 360
	GTGGACCCC ACGACGGCTA CGGGGACTTC AGGCCCTTCT TCCAGTCCCC CCTGGAGGAC 240
5	GACGUCAACA COCTUAACAC CUTUTGGUIC AMOUTICICEG TCCACGGGAC COMOUTICAAC 300
	GAGGAGCTCG ATATCTCCGA CTACTACCAG ATGAAGGTGA TCATTGAGCT CCCCATGCGG 360
	GACGGGTACO COCCCA CGGCCGGGGG AGCCGAATAG COCCCAAG 420
	TTCACCGTGG TOCACCC TTGGTTCCAG GRACAGG GGGTCCGGGT TOCACCGC 480
	CACACCTCCA TTGACCACCC CACCCCGGAG AAGTACAAGG AGGCCTACTA CTGGCACCGC 540
10	GACTGGTACG TOTOCHACTG GACCTTTGAC CCCCG AGGTGGAGAA GGCCATCCCC 600
	CACACCTCCA TTGACCACCOGAG AAGTACACCAC AGGCCTACTA CTGGCACCACCACCACCACCACCACCACCACCACCACCACCA
	GACTTTGAAA CCTCAACTGG GACAGCCTGGA CGCCTGGA CGCCCGGAGACCAT TGAGGCGGTG 720 CAGGTCATGT TCTTCTGGGC GACCTCCTGC GAGAACCTCC CCTCGCCGAG CCTCCCCCTACGCCCCC TACGGCCCCG GGAAGATCCT CCTCGCCGAG 780 AAGCGCCTGG GGAAGGCCCT GGAGGAGGC TACGGCCCCC TACGGCGGA GGACCGGGGT 840 AAGCGCCTGG GGAGGAGGA GACCCTCCCC TTCATGGCCC TAAGGCCGGA GGACCGGGGT 900
	CAGGTCATGT TCTTCCCCTGC GAGAGCCCCCG GGAAGATCCT CCTCCCCCTACGCGAGAGCCCCCC GGAGAGCCCCCC GGAGAGCCCCCC TACGGCCCCGA GACCGGGGA GGACCGGGGT 840 GCCAACATGT GCCCGGAGA GCCCCCCCC TACGCCCC TAAGGCGGA GGACCGGGGT 900 GCCAACATGT TCCCCCTGAT GCCCCGGATC GGGATCCCCC AAACCGCCCA GTGGGCCCTC 960
	TACCTCTACG AGCGGGACCT GGAGGAGCGC TACGGGGACGG GGTCCACACACACACACACACACACACACACACACACACA
15	GCCAACATGT GGCCGGAGGA GACCCCGGATC TTCATGGCCC TAAGGCCCCCA GTGGGCCCTC 960
	GCCTACACT TCCCCCTGAT GCAGGGGGG GGGATCCCCC GCAGGAGGA GCGGGAGTTC 900
	AAGCGCCTGA GGACGAGGA GACCCTCCCC TACTTCATGGCCC TAAGGCGGGA GGACCACCTC 900 GCCAACATGT GGCCGGAGGA GCCCCGGATC TTCATGGCCC AAACCGCCCA GTGGGCCCTC 960 GCCTACAACT TCCCCCTGAT GCCCGGAGGAGGA GCGGGAGGTTC 960 CCCATTGAAA CCATGCTCAA GGAGGCGGAG GAGAAGGTCA CCGCGGGAGTC CCCCCCCC 1020 CCCATTGAAA CCATGCTCAA GCTCACCCTG GAGAAGGTCA ACCTGGGGAT CCGCCGCCGC 1020 CCCATTGAAA ACCACGACGA GCTCACCCTG TTCCGCATCA ACCTGGGGAT CCCTCCTCCTC 1080
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	GGGCGCTACC GCTTGACCCT CTCCCCGAC TGGGCCGAGG ACCCCTGGTC 1800 GAGGCGGTGC TCCACCTCCC CTCCCCGAC TCCTGGTGAA GGAGAAGAGC 1800 GACCTGCCC GGGTCCACAT GCCCGGGGG CCGGAGGTCC AGACCCTGAA GGAGAAGAGC 1860 GACCTGCCC GGGGGAGGA GCTCCTTAAAC GCCCTCGCCC CCCTCCGCTT CCAGAAGGAC 1920 CACGAAAGGG GGCGGAGGA GAAGGTGGCC CTCCTGGACG GGACCCTTCCA GGTCTCCCTC 1920 CACGAAAGGG GAGAACCACA GGACCCTCCA GGTCTCCCTC 1980
	CACCTGCCCC GGGTCCACAT GCTCCTAAAC GCCCTCGCCC AGACGCCT CCAGAAGGAC 1800
30	CALGARAGG GGCGGGAGGA GARGGTGGCC CTCCTGGACG CGACCCTCCA GGTCTCCCTC 1920
	ΨCGCTCGCCC AGOM CCTCCAGCTC
	TGGCTCGCCC TCAAGCCGCT GCTCCAGCTG GAGAACCACA TCTTCGCCCG CACCCACGCC 2040 CCGCCCTTT ACCTCACCA GAGGCGGAA GGCCCCAGGCT TCTTCGCCCG CCTCCTCGC 2040 CCCCTCCTCT GGTCCCCCA GAGGCGGAA GCCCAGGCT TCTACCGCCT CCGCCACCCG 2100 CAGCCCGGCT ACTTCTACGA GCTCTCCTTC GACCCAGGCCT ACTACCGCGG GGAGGGGGTC 2160 CGCCTTAAGG AGGGGTTTGA GGGGCGGAAC CGGCCGGGAC GGACGGAGGGGGCC CCGCACCCC 2220 CGCCTTAAGG CCGAGGCCGT GGACCTCCTC CGGCCGGGAC GGTCCTCCCC 2220 CGCCCTCCTCCC CCGCCGGGAC GGACCGGACCG GGTCCTCCCC 2220 CGCCCTCCTCCC CCGCCGGGAC GGCCCTCCCC 2220 CGCCCTCCTCCC CCGCCGGGAC GGCCCTCCCC 2220 CGCCCTCCTCCC CCGCCGGGAC GGCCCTCCCC 2220 CGCCCTCCTCCC CCGCCGGAC GGCCCTCCCC 2220 CGCCCTCCTCCC CCGCCGGAC GGCCCTCCCC 2220 CGCCCTCCTCCC CCGCCCCC CCCCCCC CCCCCCCCCC
	CCCTCTCT GTTCTACGA GCTCTCCTTG GACCAGGGT ACTACGGGGG CCGCCACGTC 2160
	CAGCCCGGGGA TO CAGCCCGGAGG TO CAGCCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
3	CCCTIANO TO ACCOM GGALLICCIO TO ACCO GCALGGROUD TO ACCOCC 7280
	COTOCOTO CAAGACGO TO TOTOCO TOTOCO TOTOCO TOTOCO TOTOCO TOTOCO TOTOCOTOC
	TGGGTCCAGC TCGGCCTCGGT TCTCCGGCCC GAAGGGGGGCGC GCGCCCCCA GGACCTCTC 2400 CGCCTGGACC TCCCCGCGCT CACGGGAAGC CTCCCCCCGG TCCGGGGCA CGCCCCCGGG 2460 TCCAGAAGGG TCCTCGCCCT CCTGGAAAGC CTTCCCCGCC TCCGGGGGCCAC 2520 GCCGCCCTGG AGGTCCGGCT CCTGGAAAGC CCTGGTCGG AGGGGGGCCAC 2580 TCCTTCCCGG TCCTTCCCGG GGCCCTGCAG AGGGGGTGGT AGGGGGGGCCAC 2580
	CGCCTGGACC TACCGGGGAAGC TACCGGGGGCA COTT
	TO AGAMOO
	40 GCCCCAGGCC TCCTTCCCGG GGCCCTGT GGGGAGGTGG AGGGGGGT GTACCTCGTG 2580
	TCCAGAAGGG TCCTCGCCCT GCGCCCTGG AGGTCCGGCT CCTGGAAAGC CTTCCCCGCC CCTCCTCGGG 2520 GCCGCCCTGG AGGTCCGGCT CCTGCAC GAGACCGAAG CCCTGGTCCG GGGGGGCCAC 2520 ACCCCAGGCC TCCTTCCCA CCGGGCCCTT GGGGAGGTGG AGGGGGAGGT GTACCTCGTG 2580 GTGCGCCTCG CCCTCCTCCA CCGGGGCCTT CCGGGGCCTTC CTGGAGGTGG AGGGGGAGGT GTACCTCGTG 2640 GTGCGCCTAG GCCGCGGCCT CCGGGGCCTTC CTGGAGGAGACC TGGCCCTACGAC 2640 GTGCGCCTAG GCCGCGCCCT CCGGGGCCTTC CTGGAGGAGACC TGGCCCTTTGCC 2700
	GCCGCCTGG AGGTCCGGCT GGCCCTGCAC GAGACCGAAG CGGGGGGGCCAC 2580 ACCCCAGGCC TCCTTCCCGG GGCCCTTT GGGGAGGTGG AGGGGGGGGTGT GTACCTCGTG 2580 GTGCGCCTCG CCCTCCTCCA CCGGGCCCTT CTGGAGGTGG AGGGGGAGGT GTACCTCGTG 2640 CCCCTCCTAG GCCGCGGCCT CGGGGCCTTT CGGGCCCTCCCC 2760 CCCCTCCTAG CCGGAAAAGCG GGCCACGGTG GAGGAGGACC CGGAGCTTTG CCCCTCCCC 2760
	ACCCAGGCC TCCTCCACACCT CGGGCCCTT GGGGAGCTGG AGGGGGAGGT GTACCTCCACGC 2640 GTGCGCCTCCTAG GCCGCGGCCT CGGGGCCTTC CTGGAGGAGCTCG GGCCTACGAC 2700 GCCCTGGGGC CGGAAAAGCG GGGCACGGTG GCCCTGGAGG CCGGAGCTTTGCC 2760 GTGGAGCGGG CCGGCCTCCAAG CCTACCGCTC CCCCCCCCC 2760 GTGGAGGAGGGG CCGACCACCT CCACGCCGCC CCGACGAGGACCC CGGCCACACGC CCGACCACCT CGCCCACCACGC CGGCCACATGG CCGAGGTGGC GGCCAAGGCC 2880 45 GGGGAGGAGCA CGCCACCT CGCCCACCC CGGCCACACGC CGGCCACACGC CGGCCAAGGCC 2880 46 GGCCAAGGCC CGCCACCT CGCCCACCC CGCCCACGCC CGCCCACGCC CGCCAAGGCC 2880
	GTGGAGCGGG CCGTGCACCT CGCGCCGCC TTCCTCCAAG CCTACCGGGGGGCAC 2820
	GAGGAGGTGG CCGACCACCT CCGACGACGACGACGACGACGACGACGACGACGACGACGA
	GCCCTGGGCG CGGCACCT CGCCCTCGAG GCCCTACCGCTC CGCCCTCGAG GCGAGGAGCAC 2820 GTGGAGCGGG CCGACCACCT CCACGCCGCC TTCCTCCAAG CCTACCGCTC CGCGAGGAGCAC 2880 GAGGAGGTGG CCGACCACCT CGCCCGCAAG CGGCACATGG CCGAGGTGGC GGCCAAGGCC 2880 GAGGAGGCCC TGGAGGAGGCC CGCCCGCAAG CGCCATCCACG AGCGCTGGCA AGCGCTGCA AGCGCTGGCA AGCGCTGGCA AGCGCTGGCA AGCGCTGGCA AGCGCTGGCA AGCGCTGCA AGCGCTGGCA AGCGCTGCA AGCGCTGGCA AGCGCTGCA AGCGCTGGCA AGCGCTGCA AGCGCC AGCA AGCGCTGCA AGCGCC AGCGCA AGCGCCA AGCGCC AGCA AGCGCTGCA AGCGCC AGCA AGCGCTGCA AGCCCA AGCCA AGCCCA AGCCCA AGCCCA AGCCCA AGCCCA AGCCCA AGCCCA AGCCCA AGCCCA
	CTCCACCGG
	GGAAAAGCC

- The DNA of claim 6, wherein one or more bases in SEQ ID NO:4 are replaced with other bases by means of the degeneracy of genetic code without alternating the amino acid sequence in SEQ ID NO:3.
- B. The DNA of claim 5, which has the base sequence in SEQ ID NO:5:

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	SEQ ID NO:5: GCCCCTCCCT CCCCAACCG GGCCTTCCCG TGGGGGGGC GTGACCCCTT GCGGGCCAGG GCCCTCCCT CCCCCAACCG GCCCTTCCC GCCGTGGCC GTGACCCCTT TCGCCCTGC CCTGCACTAC CCCCTCTTGC GCCGTGGAG GACAAGGGCT TAAGGCGGTG AGCCCTGGC TAAGGCGGTGGAG GACAAGGGCT TAAGGCCGTTG AGCCCTGGC TAAGGCGGTGGAG ACAAGGGCT TAAGGCCGTTG AGCCCTGGCT TAAGGCCGTTG AGCCCTGGCT TAAGGCCGTTG AGCCCTGGCT TAAGGCCGTTG AGCCCTGGCT TAAGGCCGTTG AGCCCTGGCT TAAGGCCGTTG AGCCCTGGCT TAAGGCCGTTG AGCCCTTGCT AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT AGCCCTTGCT AGCCCTTGCT TAAGGCCGTTG AGCCCTTGCT AGCCCTT	.0
		10
5	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	20
		80
	A A GULLARO CAMPACA GONCONO	40 88
	GAGGGGCTCC TGGGGGCCTT GGGCCAAGCG GTCCTCAGGC CCGCCTTCCT CGCCTTAACGAGGCCCAAGG GGGCCAAGG CCGCCTCAAGGAAGCCCAAGG CCGCCCAAGG CCGCCCAAGG CCACACCCCCG TTTACATCGG GGACGACACC ACCGACGAGG CCGCCCAAGG CCGCCCAAGG CCACACCCCC CACACCCCCC TCACCTTCAA GGCCCCACCTAG CCTTTAGGCC GGCCGGGGCC TCACCTTCAA ACCTACCTCC GACCCACTAG CCTTTAGGCC GGCCGGGGCC ACCCTCCCACCTC CACCTCC CACCTC CACCTCC C	
10	CACCITODADO MCC TAC AND DIT	
	CTC GAC CCC	536
	Met Asp Pro Leu TTP 172 10 10 10 10 10 10 10 10 10 10 10 10 10	
	CGC TCC TTC TTT GAC GCG ASD Ala ASD ASD ASD GIY 192 30	684
15	Arg Ser Phe Phe 20 ZO TAC CTG GAG GAG CTC GGG GTC ARC Thr Leu	
	CTG AGG CGG AAG CTT CCC TAG Leu Glu Glu Leu G11 45	732
	Leu Arg Arg Lys Leu 112 40 40 GAC GAC GAC GGG TAC GALL AND	
•		780
20.	TTD Leu Met Pro Phe Phe GIN 55	, , ,
		020
	ATC TCC GAC THE TYP GIN ILE LEU 12 75	828
25	TTC ACC GTG GAC GAS Ala His Gly Arg 90 CAG GAG GCG	876
	TTC ACC GTG GAC GAG GGG GGG GGG GGG GGG GGG GGG GG	
	CTC GTC CTG AAC CAC ACC ISE ILE ASP HIS PIO ITE 110	924
	Leu Val Leu ASh 1125 105 105 TAC GTG TAC GTG TGG AGC OND Thr	
30		972
	Arg Lys Pro Asn Ser Flo 120 Arg TTC AAG GAC TTT GAA ACC	
	TO BAC GIG GIO TO THE PARTY INTE	1020
	Arg Lys Pro Asn Ser FTo 120 Arg Lys Pro Asn Ser FTo 120 CCG GAG AAG TAC AAG GGG GTC CGG GTC ATC TTC AAG GAC TTT GAA ASD CCG GAG AAG TAC AAG GGG GTC CGG GTC ATC TAC TAC TGG CAC CGC Pro Glu Lys Tyr Lys Gly Val Arg Val Ile Phe Lys Asp Phe Glu Thr 130 TCC AAC TGG ACC TTT GAC CCC GTG GCC AAG GCC TAC TAC TGG CAC CGC TCC AAC TGG ACC TTT GAC CCC GTG GCC AAG TYR TYR TYR TTP His Arg 160 TCC AAC TGG ACC TTT Phe Asp Pro Val Ala Lys Ala Tyr Tyr Trp His Arg 155 155 166 157	1020
3		
	Pro Glu Lys Tyr Lys 135 130 TCC AAC TGG ACC TTT GAC CCC GTG GCC AAG GCC TAC TAC TGG CAC TCC AAC TGG ACC TTT GAC CCC GTG GCC AAG GCC TAC TAC TGG CAC TCC AAC TGG ACC TTT GAC CCC GTG GCC AAG GCC CCC GAG GTG GAG Ser Asn Trp Thr Phe Asp Pro Val Ala Lys Ala Tyr Tyr Trp His Arg 150 Ser Asn Trp Thr Phe Asp CCC GTC AAC TGG GAC AGC CCC GAG GTG GAG Ser Asn Trp Thr Phe Asp CCC CTC AAC TGG GAC AGC CCC GAG GTG GAG 150 CAC CTC AAC TGG GAC AGC CCC GAG GTG GAG Ser Asn Trp Thr Phe Asp Pro Val Ala Lys Ala Tyr Tyr Trp His Arg 160 160 170 170 170 170 170 170	1068
	TCC AAC TGG ACC TTT SAP Pro Val Ala Lys 155 Ser Asn Trp Thr Phe Asp Pro Val Ala Lys 155 155 Ser Asn Trp Thr Phe Asp Pro Val Ala Lys 155 155 145 TTC TAC TGG CAC CAG CCC GAC CTC AAC TGG GAC AGC CCC GAG GTG GAG 145 TTC TAC TGG CAC CAG CCC GAC CTC AAC TGG GAC AGC CCC GAG GTG GAC 175 170 Phe Tyr Trp His Gln Pro Asp Leu Asn Trp Asp Ser Pro Glu Val Asp 165 TTC TGG GCC GAC CTG GGG GTG GAC	
	TTC TAC TGG CAC CAG COS Asp Leu Ash TTP ASP 170	1116
	40 Phe Tyr Trp Als 165 TTC TTC TGG GCC GAC CTG GGG Val Asp	
	TTC TAC TGG CAC CAG GAD Pro Asp Leu Ash TTP 170 40 Phe Tyr Trp His Gln Pro Asp Leu Ash TTP 170 AAG GCC ATC CAC CAG GTC ATG TTC TGG GCC GAC CTG GGG GTG GAC AAG GCC ATC CAC CAG GTC ATG TTC TTC TGG GCC GAC CTG GGG GTG GAC AAG AAA Ile His Gln Val Met Phe Phe Trp Ala Asp Leu Gly Val Asp Lys Ala Ile His Gln Val Met Phe Phe Trp Ala Asp Leu Gly Val Asp 180 180 TGC CCC TAC CTC TAC GAG CGG GAG GGG ACC	1164
	Lys Ala Ile His Gin 180	
	AAG GCC ATC CAC CAC GAI Met Phe Phe Try 190 190 Lys Ala Ile His Gln Val Met Phe Phe Try 185 180 GGC TTC CGC CTG GAC GCC ATC CCC TAC CTC TAC GAG CGG GAG GGG ACC	
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	cly Phe Arg	Leu Asp A	ala Ile Pr	o Tyr Lei	Tyr Glu Arg	Glu Gly Thr	
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							1260
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	GCG GAG GGG	ATC CCC	GAA ACC G	CC CAG TO	n Ala Leu Ph	CTC CGC AAC Leu Arg Asn	
	Ala Glu Gly	TIE PIO	295		300	CCG GAG TTC	1500
20	CAC GAC GAG	CTC ACC	CTG GAG A	AG GTC AC	or GAG GAG GAG or Glu Glu Gl	G CGG GAG TTC u Arg Glu Phe 320	
	His Asp.GIV	1 Leu III	310	-	315	- AAC CTG GGG	1548
	ATG TAC GAG	G GCC TAC	GCC CCC C	SAC CCC A	ys Phe Arg Il	C AAC CTG GGG e Asn Leu Gly 335	
	Met Tyr GI	325		3	30 . 30 CC CAC CG	C AGG CGG TAC	1596
25	ATC CGC CG	C CGC CTC	ATG CCC (Leu Leu G	ly Gly Asp Ar	C AGG CGG TAC g Arg Arg Tyr 350	
		3011				C CC AIL GIC	1644
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	AAC CAG C	AC GCC AA	G ATC TTC	GGC CGG	GGG AGC CTC A	CC CTT CTC CCC)
4 5							
	GTG GAG A	AC CGG CG	C GTC CTC	GCC TAC	Leu Arg Glu	CAC GAG GGG GAG His Glu Gly Glu 480	
	Val Glu A	sn Ary Ar	470	_	475	AG GCC TTT GAC	2028
50	CGG GTC C	TG GTG GT	G GCC AAC	CTC TCC	Arg Tyr Thr	CAG GCC TTT GAC Gln Ala Phe As 495	Þ
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CT Le	C CCC TTG GAG GCC TAC CAA GGC CTC GTC CCC GTG GAG CTC TTC ISO Leu Pro Leu Glu Ala Tyr Gln Gly Leu Val Pro Val Glu Leu Phe Ser 510 500 G CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC TTG ACC CTG GGC AG CAA CCC TTC CCC CCG GTG GAG GGG CGC TAC CGC TTG ACC CTG GGC Cla Pro Phe Pro Pro Val Glu Gly Arg Tyr Arg Leu Thr Leu Gly 525 526 527
5 CF	SUS SOU STORM SOU
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C F 5	TO His Gly Phe Ald 535 530 530 CAC CTC CCC GAC TGG GCC GAG GAG CCC GCC CCC GAG GAG GAG CAC CTC CCC TCC CCC GAC TGG GCC GAG GAG GCC GCC CCC GAG GAG GAG ALG CTC CCC CCC GAC TGG GCC GAG GAG GCC GCC GCC GAG GAG GCC S55 S50 S645 S6AC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC CTG GTG S6AC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC CTG GTG S75 S6AC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC CTG GTG S75 S70
15	SAC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC Leu Val SAC CTG CCC CGG GTC CAC ATG CCC GGG GGG CCG GAG GTC CTC Leu Val SASP Leu Pro Arg Val His Met Pro Gly Gly Pro Glu Val Leu Leu AGC CTC SASP Leu Pro Arg Val His Met Pro Gly GAG GAG CTC CTA AAC GCC CTC SASP Leu Pro Arg Val His GGG CGG GAG GAG CTC CTA AAC GCC CTC GAC ACC CTG GTC CAC GAA AGG GGG CGG GAG GAG CTC CTA AAC GCC CTC GAC ACC CTG GTC CAC GAA AGG GGG CGG GAG GAG CTC CTA AAC GCC CTC SASP Leu Pro Ser Table Val His Met Pro Gly Gly Pro Glu Val Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC GAC CTG GTC CAC GAA AGG GGG CGG GAG GAG CTC CTA AAC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC GCC CTC SASP Leu Pro Arg Val His Glu Arg Gly Arg Glu Glu Leu Leu AGC CTC CTC CTC CTC CTC CTC CTC CTC CTC C
	GAC ACC CTG GTC CAC GAA AGG GGG CGG GAG GAG CTC CTA AAC GCC GAA AAC AAC
20	ASP THE LEW 580 S80 GCC CAG ACC CTG AAG GAG AAG AGC TGG CTC GCC CTC AAG CCG CAG INC GCC CAG ACC CTG AAG GAG AAG AGC TGG CTC GCC CTC AAG CCG CAG INC GCC CAG ACC CTG AAG GAG AAG AGC CTC CGC TTP Lew Ala Lew Lys Pro Gln Lys 605 GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTT TAC GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTC TAC GTG GCC CTC CTG GAC GCC CTC CGC TTC CAG AAG GAC CCG CCC CTC CTC CTC CTC CTC CTC CTC CT
•	610 CTC CAG CTG GAG AAC CAC AGG ACC CTC CAG GIO Val Ser Leu
25	630 625 GEG TGG TCC CCC CAG AGG CGG GAA GGC CCC GGC CTC Phe Ala
	Pro Leu Bed 145 645 645 TTC TAC GAG CTC TCC TLEU ASP Pro
30	Arg Thi has 660 ctc ctc ctc ccc ccc cct AAG GAG GGG Thi Glu Gly
ar.	675 675 675 676 677 677 677 677 677 677
35	Arg Sel Led Las 695 695 CTC GGG GGG GGG GGG GGG GGG GGG GGG GGG
40	710 705 705 705 705 705 705 705 705 705 70
	725 TTP VAI GIN 725 CCC CGC CTC GAC CTC CCC TGG GTT CTC CGG CCC San Gly Gly Val Leu Arg Pro Glu Gly
45	Arg val bound 740 740 TC AGA AGG GTC CTC AGA AGG GTC CTC AGA Leu Thr
	755 755 CCC CCG GGC CCC CAG GAC CTC TTC GCC Ala Leu Glu
50	GGA AGC CTC CGG GIY Arg Pro GIN ASP 780 GIY Ser Leu Pro Pro GIY Arg Pro GIN ASP 780 770 GTC CGG CTC CTG GAA AGC CTT CCC CGC CTC CGG GGG CAC GCC CCC GGG Val Arg Leu Leu Glu Ser Leu Pro Arg Leu Arg Gly His Ala Pro Gly Val Arg Leu Leu Glu Ser Leu Pro Arg Leu Arg Gly His Ala Pro Gly

				900	2988	
	790 CA GGC CTC CTT CCC Pro Gly Leu Leu Pro 805		705	CCC CTG GTC	2900	
		0	GAG ACC GAA	Ala Leu Val		
	790	CCC CTG CAC	Glu Thr Glu	815	3036	
	TO CTT CCC	GGG GGG Leu His	5 624	COT GGG GAG	300-	
785	CA GGC CTC TEU Pro	o GIY AID BI	CAC CGG GCC	Jou Gly Glu		
ACC C	CA GGC CTC CTT CCC Pro Gly Leu Pro 805	CCC CTC CT	C Us Arg Ala	, 030 -	3084	
Thr b	CTG CG	C CTC GCC Leu Le	N HIS -	CCC CTC GGG	300	
_	CA GGC CTC CTT CCC Pro Gly Leu Leu Pro CTC CTC GGG GTG CG Leu Leu Gly Val Ar GAG GGG GTG GTG GG G1U Gly Val Val G	g Leu Ald 825	- CTA GGC CGC	Cly Leu Gly	f .	
_ CCC	Leu Gly Va-	THE CAC CCC CT	IC CIN GLY AT	g Gri -	3132	
Arg	GAG GGG GTG GGG GIU GIY Val GB35 TTC CTG GAG CTG CTG GAG CTG GAG CTG CTG GAG CTG CTG CTG CTG CTG CTG CTG CTG CTG CT	G GGC UNS Pro L	eu Bes 84	5 cmc GGC GCC	<i>j</i>	
	CAG GGG GTG Wal G	ly Gly RAO	GTG GC	C Clo Gly Al	a	
GTG	Clu Gly Val Val	GAG GTG T	AC CIO Val A	ra nec	3180	
Val	835 - CTG G	AG GGG GAU Val	LAT 1960 "	-C CCC TAC GA	,C 0-	
_	MTC CTG GAG LAU (31u GIY GI	GCC CGC C'	TG GOO TYT A	5 p	
CCC	phe Leu GIU Bes	855 GAG GAC	CTG SUL AIG L	eu na	3228	
Ala	GAG GGG GTG GTG GG Glu Gly Val Val G 835 TTC CTG GAG CTG G TTC CTG GAG CTG G B50 A AAG CGG GGC ACG A AAG CGG GGC ACG	GTG GAG GIU ASP	Leu 875	-sc GCG GAG C	TT	
	AAG CGG GGG Thr	Val Gro	CC CTG	Ala Glu I	ما عال	
GA	. LVS ATG GIY	870 GCC CTC	GAG Jala Leu	895	3276	
; G1	835 C TTC CTG GAG CTG G Phe Leu Glu Leu G 850 A AAG CGG GGC ACG LU Lys Arg Gly Thr 65 TG GAG CGG GCC GTG al Glu Arg Ala Val 885 TGG GCC TTT GCC GAG TTT GCC GAG TTT Ala Phe Ala Glu	CAC CTC Ala Leu	GIU	TOC GCC TTC	J'I'C	
80	nc GAG CGG GGG Val	His Dec	CTC CAC	Ala Phe	Den	
G.	al Glu Arg Ala 885	GCC GAC	CAC Leu His	910	3324	4
V	acc GAG	GAG GIG Ala ASI	p H15	CAG GAG GCG	63.77	
_	ACC GCC TTT GCC GI	a Glu Var 90	5 CAG GCC CTG	Clu Glu Ala	GIY	_
20	GS GAG CGG GCC GTG RG GAG CGG GCC ATG RGG GCC TTT GCC GAG RTP Ala Phe Ala GIV RTP Ala Phe Ala GIV CAA GCC TAC CGC TC GIN Ala TYX ATG SE GIN ALA GCC AC	- CTC CCC GA	G GAO Ala Leu	025	CAG 337	2
7.	TIP TO GEC TO	C GCC CIO Pro GI	au Gra	CTC CAC CGG	GIN	
	CAA GCC TAC SE	IT Ala lies 920	TO GCG GAG CAL	Leu His Arg	, 02-	
	GIR Ala Tyr Ala	TOC GAG GTG G	Ala Glu Hi	5 500	- GCC 34	20
	915 CAC A'	re GLC Glu Val A	10 / 94	C CAG GCC AAL	~ Ala	c
25	CAA GCC TAC CGC TCGIN Ala TYP AIG SEGIN ALA TYP AIG SEGIN ALA TYP AIG SEGIN ALA TYP AIG SEGIN ALA TYP THE AIG HIS MORE AGE CCC GCC GAA AGG CCC GCC GAA AGG CCC GCC G	et Ala 935	TO GAG CGC TG	Gln Ala Ly	960	
	TIP THI ALY	- NG CGC ATC	He Glu Arg TI	-p	34	129
		Tare ALM	-			
	GAA AGG CCC Ala	Arg LYS		•	_	400
	Glu Arg Plo	950		- 30	ACGGCCTC 3	1407 1510
	945		1	TTGGGGTGGA AG	CGGCAGAA	30 4 2
30	GGA AAA GCC	- 50	CACGGGGGCC .	CGTCCCACA	,	3000
	GGA AAA Ala Gly Lys 963	AGCCCCGGG	C GCGGTAGACG	CACTCCCTAA		
	-CCCG GTAG	ACCCTTC AGCCCCGGGGGGGGGGGGGGGTTCTTGGCCC	C GCACCGCTCG	J		
	TAGGCGCCAGG AGGC	TGGGGTAGL	,0			
•	CTCGGGGGACC GCCC	CCG1GG	و ياد م 15	enus Thermus.		
35	GGCGCACACC		croorganism of the go		- a the enzyme (oi cia

- 9. The DNA of claim 5, which is derived from a microorganism of the genus Thermus.
- 10. A replicable recombinant DNA which contains a self-replicable vector and a DNA encoding the enzyme of claim 1. 40
 - 11. The replicable recombinant DNA of claim 10, wherein said DNA contains a base sequence selected from the group The replicable recombinant DINA of Claim 10, wherein said DINA contains a base sequence selected from the group consisting of the base sequence in SEQ ID NO:4, homologous base sequences thereunto, and complementary
 - 12. The replicable recombinant DNA of claim 11, wherein said DNA is obtained by replacing one or more bases in SEQ The replicable recombinant DINA of claim 11, wherein said DNA is obtained by replacing one or more bases in SEQ ID NO:4 with other bases by means of the degeneracy of genetic code without alternating the amino acid sequence is SEQ ID NO:3 45
 - 13. The replicable recombinant DNA of claim 10, wherein said DNA has the base sequence in SEQ ID NO:5.
 - 14. The replicable recombinant DNA of claim 10, wherein said DNA is derived from a microorganism of the genus
 - 15. The replicable recombinant DNA of claim 10, wherein said self-replicable vector is plasmid vector Bluescript II SK
 - 16. A transformant which is prepared by introducing into an appropriate host a replicable recombinant DNA which con-

tains a DNA encoding the enzyme of claim 1 and a self-replicable vector.

- 17. The transformant of claim 16, wherein said DNA has a base sequence selected from the group consisting of the the manifestration of claim 10, whistern said DIAC has a base sequences thereunto, and complementary base sequences to base sequence in SEQ ID NO:4, homologous base sequences thereunto.
- 18. The transformant of claim 17, wherein the said DNA is obtained by replacing one or more bases in the base sequence in SEQ ID NO:4 with other bases by means of the degeneracy of genetic code without alternating the amino acid
- 19. The transformant of claim 16, wherein said DNA has the base sequence in SEQ ID NO:5.
- 20. The transformant of claim 16, wherein said DNA is derived from a microorganism of the genus Tharmus.
- 21. The transformant of claim 16, wherein said self-replicable vector is plasmid vector Bluescript II SK(+) or pKK223-3.
- 22. The transformant of claim 16, wherein said host is a microorganism of the species Escherichia coli.
- 23. A process for preparing a recombinant enzyme, which comprises:

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- culturing a transformant, prepared by introducing into an appropriate host a recombinant DNA containing both a self-replicable vector and a DNA encoding the recombinant enzyme of claim 1, in a nutrient culture medium
- 24. The process of claim 23, wherein said DNA has a base sequence selected from the group consisting of the base collecting the formed enzyme from the resultant culture. sequence in SEQ ID NO:4, homologous base sequences thereunto, and complementary base sequences to these
- 25. The process of claim 24, wherein the said DNA is obtained by replacing one or more bases in SEQ ID NO:4 with other bases by means of the degeneracy of genetic code without alternating the amino acid sequence in SEQ ID 30
 - 26. The process of claim 23, wherein said DNA has the base sequence in SEQ ID NO:5.
 - 27. The process of claim 23, wherein said DNA is derived from a microorganism of the genus Thermus.
 - 28. The process of claim 23, wherein said self-replicable vector is plasmid vector Bluescript II SK(+) or pKK223-3.
 - 29. The process of claim 23, wherein said host is a microorganism of the species Escherichia coli. 40
 - 30. The process of claim 23, wherein the recombinant enzyme formed in the nutrient culture medium is recovered by centrifugation, filtration, concentration, sating out, dialysis, separatory sedimentation, ion-exchange chromatography, gel filtration chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and/or
 - 31. An enzymatic conversion method of maltose, which comprises a step of allowing the recombinant enzyme of claim
 - 32. The method of claim 31, wherein the step comprises coexisting an effective amount of the recombinant enzyme in an aqueous medium containing maltose up to 50 w/w %, and subjecting the resultant mixture to an enzymatic 50
 - 33. The method of claim 31, wherein the resulting reaction mixture contains at least about 50 w/w % trehalose, on a dry solid basis. 55

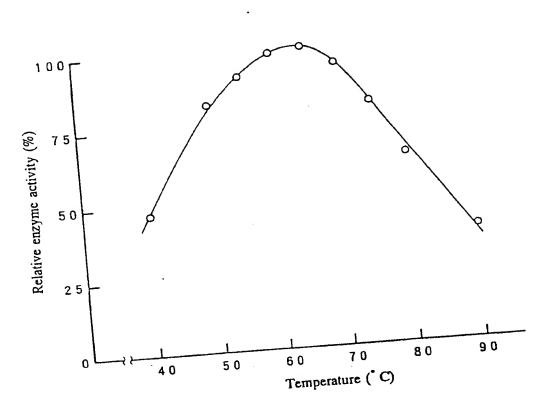


FIG.1

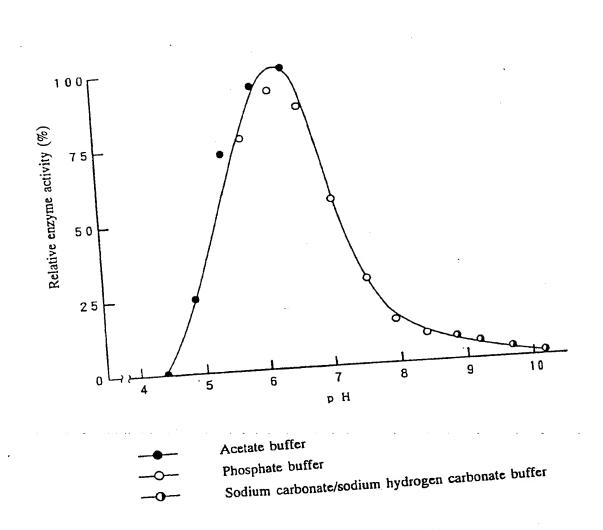


FIG.2

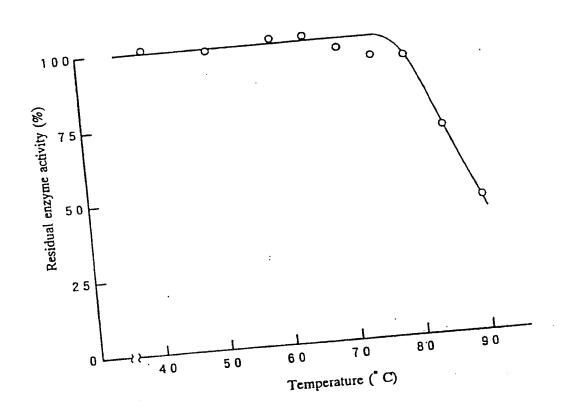


FIG.3

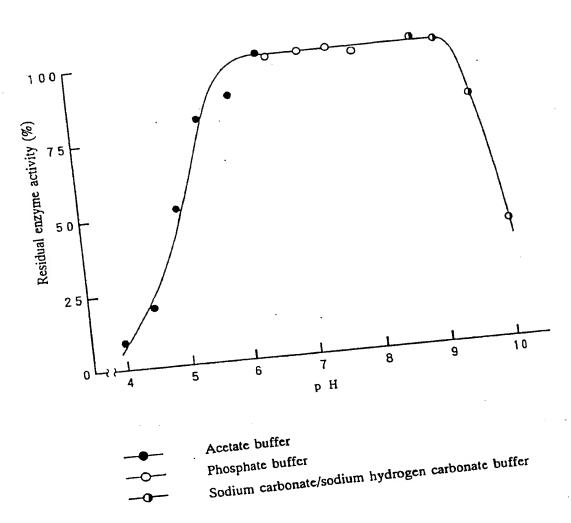


FIG.4

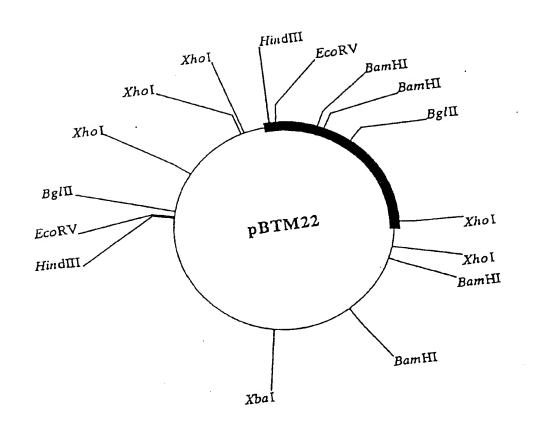


FIG.5